

**CONJOINT ADMINISTRATION OF MORPHOGENS AND ACE  
INHIBITORS IN TREATMENT OF CHRONIC RENAL FAILURE**



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10    **Related Applications**

This application claims priority to the filing date of U.S. Provisional Application No. 60/406,431, filed August 28, 2002, entitled "Conjoint Administration of Morphogens and ACE Inhibitors In Treatment of Chronic Renal Failure," the entire teachings of which are hereby incorporated by reference.

15    **Background of the Invention**

The mammalian renal system serves primary roles both in the removal of catabolic waste products from the bloodstream and in the maintenance of fluid and electrolyte balances in the body. Renal failures are, therefore, life-threatening conditions in which the build-up of catabolites and other toxins, and/or the 20 development of significant imbalances in electrolytes or fluids, may lead to the failure of other major organs systems and death. As a general matter, renal failure is classified as "acute" or "chronic." As detailed below, the differences between these two conditions are not merely a matter of severity or rapidity but, rather, reflect differences in etiology, prognosis, and treatment.

25    **Acute renal failure:**

Acute renal failure is defined as an abrupt cessation or substantial reduction of renal function and, in as many as 90-95% of cases, may be secondary to trauma, surgery or another acute medical condition. Acute renal failure may be due to pre-

renal causes (e.g., decreased cardiac output, hypovolemia, altered vascular resistance) or to post-renal causes (e.g., obstructions or constrictions of the ureters, bladder or urethra) which do not directly involve the kidneys and which, if treated quickly, will not entail significant loss of nephrons or other damage to the kidneys.

5     Alternatively, acute renal failure may be due to intrinsic renal causes which involve a more direct insult or injury to the kidneys, and which may entail permanent damage to the nephrons or other kidney structures. Intrinsic causes of acute renal failure include but are not limited to infectious diseases (e.g., various bacterial, viral or parasitic infections), inflammatory diseases (e.g., glomerulonephritis, systemic

10    lupus erythematosus), ischemia (e.g., renal artery occlusion), toxic syndromes (e.g., heavy metal poisoning, side-effects of antimicrobial treatments or chemotherapy), and direct traumas.

The diagnosis and treatment of acute renal failure is as varied as its causes. In human patients, oliguria (urine output < 400 ml/day) or anuria (urine output < 50 ml/day) may be present in 50-70% of cases, BUN levels may climb 10-20 mg/dL/day or faster, plasma creatinine levels may climb 0.5-1.0 mg/dL/day, and metabolic acidosis is almost always present. If not treated, the electrolyte and fluid imbalances (e.g., hyperkalemia, acidosis, edema) associated with acute renal failure may lead to life-threatening arrhythmia, congestive heart failure, or multiple organ system failures. Present therapies are typically directed at the underlying causes of the acute renal failure(e.g., pre-renal, post-renal, or infectious causes) and management of the complications. Due to the severity of acute renal failure, episodes rarely last longer than several weeks without mortality and are treated on an in-patient basis.

25        Chronic renal failure:

Chronic renal failure (CRF) is the progressive loss of kidney function. The kidneys attempt to compensate for renal damage by hyperfiltration (excessive straining of the blood) within the remaining functional nephrons (filtering units that consist of a glomerulus and corresponding tubule). Over time, hyperfiltration causes 30 further loss of function.

Chronic loss of function causes generalized wasting (shrinking in size) and progressive scarring within all parts of the kidneys. In time, overall scarring obscures the site of the initial damage. Yet, it is not until over 70% of the normal combined function of both kidneys is lost that most patients begin to experience 5 symptoms of kidney failure. Thus, chronic renal failure may be defined as a progressive, permanent and significant reduction of the glomerular filtration rate (GFR) due to a significant and continuing loss of nephrons.

Chronic renal failure typically begins from a point at which a chronic renal insufficiency (i.e., a permanent decrease in renal function of at least 50-60%) has 10 resulted from some insult to the renal tissues which has caused a significant loss of nephron units. The initial insult may or may not have been associated with an episode of acute renal failure. Irrespective of the nature of the initial insult, chronic renal failure manifests a "final common path" of signs and symptoms as nephrons are progressively lost and GFR progressively declines. This progressive 15 deterioration in renal function is slow, typically spanning many years or decades in human patients, but seemingly inevitable.

The early stage of chronic renal failure typically begins when GFR has been reduced to approximately one-third of normal (e.g., 30-40 ml/min for an average human adult). As a result of the significant nephron loss, and in an apparent 20 "attempt" to maintain the overall GFR with fewer nephrons, the average single nephron GFR (SNGFR) is increased by adaptations of the remaining nephrons. at both the structural and functional level. One structural manifestation of this adaptation, readily detectable by microscopic examination of biopsy samples, is a "compensatory hypertrophy" of both the glomeruli and the tubules of the kidney, a 25 process which literally increases the volume of filtrate which can be produced by each remaining nephron by literal enlargement of the glomeruli and tubules. Indeed, as a result of the hypertrophy or dilation of the collecting ducts, the urine of subjects with chronic renal failure often contains broad "casts," typically 2-6 times normal diameter, which aid in diagnosis and have also been referred to as "renal failure 30 casts." At the same time, there are functional changes in the remaining nephrons, such as decreased absorption or increased secretion of normally excreted solutes,

which may be responses to hormonal or paracrine changes elsewhere in the body (e.g., increasing levels of parathyroid hormone (PTH) in response to changes in serum levels of calcium and phosphate).

These adaptations in early stage chronic renal failure are not successful in 5 completely restoring GFR or other parameters of renal function and, in fact, subject the remaining nephrons to increased risk of loss. For example, the increased SNGFR is associated with mechanical stresses on the glomerulus due to hyper-tension and hyper-perfusion. The loss of integrity of podocyte juncitures leads to increased permeability of the glomerulus to macromolecules or "leakiness" of the glomerular 10 capsule. Proliferative effects are also observed in mesangial, epithelial and endothelial cells, as well as increases in the deposition of collagen and other matrix proteins. Sclerosis of both the glomeruli and tubules is another common symptom of the hypertrophied nephrons and the risk of coagulation in the glomerulus is increased. In particular, these adaptations of the remaining nephrons, by pushing the 15 SNGFR well beyond its normal level, actually decrease the capacity of the remaining nephrons to respond to acute changes in water, solute, or acid loads and, therefore, actually increase the probability of additional nephron loss.

As chronic renal failure progresses, and GFR continues to decline to less than 10% of normal (e.g., 5-10 mL/min), the subject enters end-stage renal disease 20 (ESRD). During this phase, the inability of the remaining nephrons to adequately remove waste products from the blood, while retaining useful products and maintaining fluid and electrolyte balance, leads to a rapid decline in which many organ systems, and particularly the cardiovascular system, may begin to fail. For example, BUN and creatinine levels may be expected to rise and, at BUN levels of 25 60-100 mg/dL and serum creatinine levels of 8-12 mg/dL, a uremic syndrome will typically develop in which the kidneys can no longer remove the end products of nitrogen metabolism. At this point, renal failure will rapidly progress to death unless the subject receives renal replacement therapy (i.e., chronic hemodialysis, continuous peritoneal dialysis, or kidney transplantation).

30 Approximately 600 patients per million receive chronic dialysis each year in the United States, at an average cost approaching \$60,000-\$80,000 per patient per

year. Of the new cases of end-stage renal disease each year, approximately 28-33% are due to diabetic nephropathy (or diabetic glomerulopathy or diabetic renal hypertrophy), 24-29% are due to hypertensive nephrosclerosis (or hypertensive glomerulosclerosis), and 15-22% are due to glomerulonephritis.

- 5       The 5-year survival rate for all chronic dialysis patients is approximately 40%, but for patients over 65, the rate drops to approximately 20%.

Morphogens and Growth Factors:

- A great many proteins have now been identified which appear to act as morphogenetic or growth factors, regulating cell proliferation or differentiation.
- 10     Typically these growth factors exert their effects on specific sets or subsets of cells or tissues. Thus, for example, epidermal growth factors, nerve growth factors, fibroblast growth factors, various hormones, and many other proteins inducing or inhibiting cell proliferation or differentiation have been identified and shown to affect some subgroup of cells or tissues.
- 15     One group of morphogenetic proteins, referred to herein as "morphogens," includes members of the family of osteogenic proteins/bone morphogenetic proteins (OP/BMPs) which were initially identified by their ability to induce ectopic, endochondral bone morphogenesis.
- Subsequent characterization of the nucleic acid and amino acid sequences of the BMPs has shown them to be a subgroup of the TGF- $\beta$  superfamily of growth factors. Members of this morphogen family have now been shown to include the mammalian osteogenic protein-1 (OP-1, also known as BMP-7), osteogenic protein-2 (OP-2), osteogenic protein-3 (OP-3), BMP-2 (also known as BMP-2A or CBMP-2A), BMP-3, BMP-4 (also known as BMP-2B or CBMP-2B), BMP-5, BMP-6, Vgr-1, and GDF- 1, as well as the Xenopus homologue Vgl and the Drosophila homologues DPP and 60A. Members of this family encode secreted polypeptides, that share common structural features and that are similarly processed from pro-proteins to yield carboxy terminal mature proteins having a conserved pattern of cysteines. The active forms of these proteins are either disulfide-bonded homodimers of a single family member, or heterodimers of two different members

(see, e.g., Massague (1990) *Annu. Rev. Cell Biol.* 6:597; Sampath, et al., (1990) *J. Biol. Chem.* 265:13198).

The members of the morphogen family of proteins are expressed in a variety of tissues during development. BMP-3 for, example, has been shown to be  
5 expressed in developing human lung and kidney (Vukicevic et al. (1994) *J. Histochem. Cytochem.* 42:869-875), BMP-4 has been shown to be expressed in the developing limbs, heart, facial processes and condensed mesenchyme associated with early whisker follicles in embryonic mice (Jones, et al. (1991) *Development* 111: 531-542), and OP-1 (BMP-7) has been shown immunohistochemically to be  
10 associated with basement membranes in human embryos, including those of the developing lungs, pancreas, skin, and convoluted tubules of kidneys (Vukicevic, et al. (1994) *Biochem. Biophys. Res. Commun.* 198: 693-700). Some of the morphogens (e.g., OP-2 and BMP-2) were not detected in analyses of adult tissues, suggesting only an early developmental role for these morphogens (Ozkaynak, et al.  
15 (1992) *J. Biol. Chem.* 267: 25220-25227). In contrast, high levels of murine OP-1 expression have been observed in adult mouse kidneys (Ozkaynak, et al. (1991) *Biochem. Biophys. Res. Commun.* 179: 116-123). This suggests a possible role for OP-1 synthesized in the kidney as a paracrine regulator of bone growth, and would be consistent with the role of the kidneys in both calcium regulation and bone  
20 homeostasis.

A great variety of growth factors have been considered which may participate in the regulation of the growth and repair of renal tissues (reviewed in, e.g., Toback (1992) *Kidney Intl.* 41: 226-246). For example, EGF, TGF- $\alpha$ , TGF- $\beta$ , IGF-I, IGMI, PDGF, FGF, Renin / Angiotensin II, IL-1 and OP-1 have all been  
25 found to be expressed by various adult renal cells or tissues and to have effects on renal cell proliferation or differentiation (see, Toback (1992) supra, Ozkaynak, et al. (1991) supra ). In addition, several of these have been found to be expressed in the developing kidney, including IGF-I, TGF- $\beta$  and OP-1 (reviewed in, e.g., Bard, et al. (1994) *Mech. Development* 48: 3-11).

30 Interestingly, TGF- $\beta$  has been shown in a murine metanephric organ culture system to retard overall growth and segmental differentiation of all segments of

developing nephrons except the thick ascending limb-early distal tubules (Avner and Sweeney (1990) *Pediatr. Nephrol.* 4: 372-377). In addition, TGF- $\beta$  expression has been found to be increased in several models of renal disease, suggesting that TGF- $\beta$  mediated increases in the synthesis of extracellular matrix components may be involved in the etiology of diabetic nephropathy (or diabetic glomerulopathy or diabetic renal hypertrophy), renal fibrosis, glomerulosclerosis and glomerulonephritis, interstitial fibrosis, and hypertensive nephrosclerosis (Shankland, et al. (1994) *Kidney Intl.* 46: 430-442; Yamamoto, et al. (1994) *Kidney Intl.* 45:916-927; Yamamoto, et al. (1993) *PNAS* 90: 1814 Tamaki, et al. (1994) *Kidney Intl.* 45:525-536; Border, et al. (1990) *Nature* 346: 371-374; Hamaguchi, et al. (1995) *Hypertension* 26: 199-207).

Also of interest is the fact that serum levels of human growth hormone (GH) are elevated in subjects with chronic renal failure (Wright et al. (1968) *Lancet* 2: 798; Samaan and Freeman (1970) *Metabolism* 19: 102). Recombinant GH has been shown to help maintain protein balance in malnourished chronic renal failure patients, and to promote "catch-up" growth in children with chronic renal failure. It has been suggested that these effects are mediated by IGF-I (see, e.g., Kopple (1992) *Miner. Electrolyte Metab.* 18: 269-275). Although some studies have found that the administration of IGF-I increases renal plasma flow and GFR in chronic renal failure patients (e.g., Guler, et al. (1989) *PNAS* 86:2868-2872; Hirschberg, et al. (1993) *Kidney Intl.* 43:387-397), other studies have found that this effect is merely transient (Miller, et al. (1994) *Kidney Intl.* 46: 201-207).

Thus, although some growth factors have been shown to be expressed in both developing and adult renal tissues, and although at least one has been shown to increase renal function in the short term, none has yet been shown to be of therapeutic benefit in preventing, inhibiting, or delaying the progressive loss of renal function that characterizes chronic renal failure. A need remains, therefore, for treatments which will prevent the progressive loss of renal function which causes hundreds of thousand of patients to become dependent upon chronic dialysis, and which results in the premature deaths of tens of thousands each year.

**Summary of the Invention**

The present invention is directed to methods of treatment, and pharmaceutical preparations for use in the treatment, of vertebrate subjects (preferably mammalian subjects) in, or at risk of, chronic renal failure, or at risk of

5 the need for renal replacement therapy by using a combination of an Angiotensin-Converting Enzyme inhibitor (ACEI) and a morphogen. Suitable subjects include subjects already afflicted with chronic renal failure, or which have already received renal replacement therapy, as well as any subject reasonably expected to suffer a progressive loss of renal function associated with progressive loss of functioning

10 nephron units. Whether a particular subject is at risk is a determination which may routinely be made by one of ordinary skill in the relevant medical or veterinary art. Subjects in, or at risk of, chronic renal failure, or at risk of the need for renal replacement therapy, include but are not limited to the following: subjects which may be regarded as afflicted with chronic renal failure, end-stage renal disease,

15 chronic diabetic nephropathy, hypertensive nephrosclerosis, chronic glomerulonephritis, hereditary nephritis, and/or renal dysplasia; subjects having a biopsy indicating glomerular hypertrophy, tubular hypertrophy, chronic glomerulosclerosis, and/or chronic tubulointerstitial sclerosis; subjects having an ultrasound, MRI, CAT scan, or other non-invasive examination indicating renal

20 fibrosis; subject having an unusual number of broad casts present in urinary sediment; subjects having a GFR which is chronically less than about 50%, and more particularly less than about 40%, 30% or 20%, of the expected GFR for the subject; human male subjects weighing at least about 50 kg and having a GFR which is chronically less than about 50 ml/min, and more particularly less than about 40 ml/min, 30 ml/min or 20 ml/min; human female subjects weighing at least about 40 kg and having a GFR which is chronically less than about 40 mL/min, and more particularly less than about 30 mL/min, 20 ml/min or 10 ml/min; subjects possessing a number of functional nephron units which is less than about 50%, and more particularly less than about 40%, 30% or 20%, of the number of functional nephron

25 units possessed by a healthy but otherwise similar subject; subjects which have a single kidney; and subjects which are kidney transplant recipients.

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The methods and compositions of this invention capitalize in part upon the discovery that certain morphogens of eukaryotic origin and inhibitors of ACE (Angiotensin-Converting Enzyme) may be used conjointly as therapeutic agents in the treatment of subjects at risk, as defined herein, of chronic renal failure or the  
5 need for renal replacement therapy. Generally, these proteins are members of the osteogenic protein/bone morphogenetic protein (OP/BMP) family of proteins or analogs thereof. In a preferred embodiment, the ACE inhibitor is enalapril.

Thus, useful OP/BMP morphogens of the invention include polypeptides, or functional variants of polypeptides, comprising at least the C-terminal six- or seven-  
10 cysteine domain of a mammalian protein selected from OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, BMP9, and proteins which exhibit at least 70% or, more preferably, 75% or 80%, 85%, 90%, 95%, 99% amino acid sequence homology, or at least 50% identity, more preferably 55%, 60%, 65%, 70%, 80%, 90%, 99% or more identity, with the amino acid sequence of the seven-cysteine  
15 domain of any of the morphogens described above, such as human OP-1; and which are (a) capable of inducing chondrogenesis in the Reddi-Sampath ectopic bone assay (Sampath and Reddi (1981), Proc. Natl. Acad. Sci. USA 78: 7599-7603) or a substantially equivalent assay, (b) capable of significantly preventing, inhibiting, delaying or alleviating the progressive loss of renal function in a standard animal  
20 model of chronic renal failure, or (c) capable of causing a clinically significant improvement in a standard marker of renal function when administered to a mammal in, or at risk of, chronic renal failure. More generally speaking, the invention provides for the use of "morphogens" which are dimeric proteins that induce morphogenesis of one or more eukaryotic (e.g., mammalian) cells, tissues or organs.

25 Of particular interest herein are morphogens that induce morphogenesis at least of mammalian renal tissue, including formation of functional renal epithelium and, in particular, functional glomerular and tubular epithelium. Morphogens comprise a pair of polypeptides that, when folded, adopt a configuration suitable for the resulting dimeric protein to elicit morphogenetic responses in cells and tissues  
30 displaying receptors specific for said morphogen. That is, morphogens generally induce all of the following biological functions in a morphogenically permissive

environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells.

"Progenitor" cells are uncommitted cells that are competent to differentiate into one or more specific types of differentiated cells, depending on their genomic repertoire and the tissue specificity of the permissive environment in which morphogenesis is induced. Morphogens further can delay or mitigate the onset of senescence- or quiescence-associated loss of phenotype and/or tissue function. Morphogens still further can stimulate phenotypic expression of differentiated cells, including expression of metabolic and/or functional, e.g., secretory, properties thereof. In addition, morphogens can induce redifferentiation of committed cells under appropriate environmental conditions. As noted above, morphogens that induce proliferation and/or differentiation at least of mammalian renal tissue, and/or support the growth, maintenance and/or functional properties of mammalian nephrons, are of particular interest herein.

In preferred embodiments, the pair of morphogen polypeptides have amino acid sequence search comprising a sequence that shares a defined relationship with an amino acid sequence of a reference morphogen. Herein, preferred morphogen polypeptides share a defined relationship with a sequence present in morphogenically active human OP-1. However, anyone or more of the naturally occurring or biosynthetic sequences disclosed herein similarly could be used as a reference sequence. Preferred morphogen polypeptides share a defined relationship with at least the C-terminal six cysteine domain of human OP-1, residues 43-139 of SEQ ID NO: 1. Preferably, morphogen polypeptides share a defined relationship with at least the C-terminal seven cysteine domain of human OP-1, residues 38-139 of SEQ ID NO: 1. That is, preferred morphogen polypeptides in a dimeric protein with morphogenic activity each comprise a sequence that corresponds to a reference sequence or is functionally equivalent thereto.

Functionally equivalent sequences include functionally equivalent arrangements of cysteine residues disposed within the reference sequence, including amino acid insertions or deletions which alter the linear arrangement of these

cysteines, but do not materially impair their relationship in the folded structure of the dimeric morphogen protein, including their ability to form such intra- or inter-chain disulfide bonds as may be necessary for morphogenic activity.

Functionally equivalent sequences further include those wherein one or more 5 amino acid residues differ from the corresponding residues of a reference morphogen sequence, e.g., the C-terminal seven cysteine domain (or "skeleton") of human OP-1, provided that this difference does not destroy morphogenic activity. Accordingly, conservative substitutions of corresponding amino acids in the reference sequence are preferred. Amino acid residues that are "conservative 10 substitutions" for corresponding residues in a reference sequence are those that are physically or functionally similar to the corresponding reference residues, e.g., that have similar size, shape, electric charge, chemical properties including the ability to form covalent or hydrogen bonds, or the like. Particularly preferred conservative substitutions are those fulfilling the criteria defined for an "accepted point mutation" 15 in Dayhoff et al. (1978), 5 Atlas of Protein Sequence and Structure, Suppl. 3, ch. 22 (pp. 354-352), Nad. Biomed. Res. Found., Washington, D.C. (see below), the teachings of which are incorporated by reference herein.

In certain embodiments, a polypeptide suspected of being functionally equivalent to a reference morphogen polypeptide is aligned therewith using the 20 method of Needleman, et al. (1970), J. Mol. Biol. 48: 443-453, implemented conveniently by computer programs such as the Align program or other improved successors / variants (DNAStar, Inc.). For example, the MegAlign program of the Lasergene 5.0 (DNAStar, Inc.) offers several multi-sequence alignment methods (J. Hein method, see Hein, J.J. (1990). "Unified approach to alignment and 25 phylogenies." In Methods in Enzymology, Vol. 183: pp. 626-645; Clustal V method, see Higgins, D.G. and P.M. Sharp (1989). "Fast and sensitive multiple sequence alignments on a microcomputer." CABIOS, Vol. 5, No. 2: pp. 151-153; Clustal W method, see J.D. Thompson, et al. (1994). Nucleic Acids Research, Vol 30 22, pp. 4673-80), each with different algorithms, and each offers user opportunities to define parameters such as gaps. Specifically, gaps and insertions are arranged to achieve the highest degree of correlation between the amino acids of the two

sequences being compared, with user specified penalties – the so-called Gap Penalty (the amount deducted from the alignment score for each gap in the alignment. Gaps of different sizes carry the same penalty), and the Gap Length Penalty (the value deducted from the alignment score after first multiplying it by the length of gaps).

- 5 Longer gaps have a greater penalty than shorter gaps). The aligned sequences will then be used to calculate a percent identity (homology) between the candidate and reference sequences. Sequence homology between a section of the aligned sequences can also be generated. In a preferred embodiment, each amino acid of a gap or insertion counts as a mismatch for measuring % identity purpose.

- 10 Of particular interest herein are morphogens, which, when provided to the kidney of a mammal, induce or maintain the normal state of differentiation and growth of nephron units. Of still more particular interest herein are morphogens which, when administered to a mammal, prevent, inhibit or delay the development of compensatory hypertrophy, including glomerular hypertrophy and/or tubular hypertrophy. Such morphogens can be used to treat a mammal in, or at risk of, chronic renal failure by preventing, inhibiting or delaying the progressive loss of functional nephron units and the consequent progressive loss of renal function.
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- The present invention alternatively can be practiced with methods and compositions comprising a morphogen stimulating agent or morphogen inducer in lieu of a morphogen. A "morphogen inducer" is a compound that stimulates *in vivo* production, e.g., expression, of a therapeutically effective concentration of an endogenous morphogen in the body of a mammal sufficient to regenerate or maintain renal tissue and/or to inhibit additional loss thereof. Such compounds are understood to include substances which, when administered to a mammal, act on cells of tissue(s) or organ(s) that normally are competent to produce and/or secrete a morphogen encoded within the genome of the mammal, and which cause the endogenous level of the morphogen in the mammal's body to be altered. Endogenous or administered morphogens can act as endocrine, paracrine or autocrine factors. That is, endogenous morphogens can be synthesized by the cells in which morphogenetic responses are induced, by neighboring cells, or by cells of a distant tissue, in which circumstances the secreted endogenous morphogen is
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transported to the site of morphogenesis, e.g., by the individual's bloodstream. In preferred embodiments, the agent stimulates expression and/or secretion of an endogenous morphogen so as to increase amounts thereof in renal tissues.

In still other embodiments, an agent which acts as an agonist of a morphogen receptor may be administered instead of the morphogen itself. An "agonist" of a receptor means a compound which binds to the receptor and for which such binding has a similar functional result as binding of the natural, endogenous ligand of the receptor. That is, the compound must, upon interaction with the receptor, produce the same or substantially similar transmembrane and/or intracellular effects as the endogenous ligand. Thus, an agonist of a morphogen receptor binds to the receptor and such binding has the same or a similar functional result as morphogen binding (e.g., induction of morphogenesis). The activity or potency of an agonist can be less than that of the natural ligand, in which case the agonist is said to be a "partial agonist," or it can be equal to or greater than that of the natural ligand, in which case it is said to be a "full agonist." Thus, for example, a small peptide or other molecule which can mimic the activity of a morphogen in binding to and activating the morphogen's receptor may be employed as an equivalent of the morphogen. Preferably the agonist is a full agonist, but partial morphogen receptor agonists may also be advantageously employed. Methods of identifying such agonists are known in the art and include assays for compounds which induce morphogen-mediated responses (e.g., induction of differentiation of metanephric mesenchyme, induction of endochondral bone formation, and the like). Such an agent may also be referred to as a morphogen "mimic," "mimetic," or "analog."

The OP/BMP morphogens, or the morphogen inducers / agonists of morphogen receptors, or ACEI of the invention, may be administered by any route of administration which is compatible with the selected agent, and may be formulated with any pharmaceutically acceptable carrier appropriate to the route of administration. Preferred routes of administration are parenteral and, in particular, intravenous, intraperitoneal, and renal intracapsular. Treatments are also preferably conducted over an extended period on an out-patient basis. Daily dosages of the morphogens are expected to be in the range of about 0.01-1000 µg/kg body weight,

and more preferably about 10-300 µg/kg body weight, although precise dosages will vary depending upon the particular therapeutic agent employed and the particular subject's medical condition and history.

Finally, in yet further embodiments, renal cells may be implanted into the  
5 kidney of a subject in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy, in order to serve as a source of morphogen and/or to provide a source of additional functional renal tissue. Preferably, the cells are induced to undergo metanephric differentiation by treatment with a morphogen (e.g., OP-1) either before or after implantation.

10 These cells may be renal mesenchymal progenitor cells, or renal mesenchymal progenitor cells which have been induced to undergo metanephric differentiation. The cells may be derived from a donor (e.g., a tissue-type matched donor, sibling, identical twin), or may be derived from a tissue culture (e.g., undifferentiated renal mesenchyme culture, fetal renal tissue culture), or may be  
15 explanted from the subject and then be re-implanted after proliferation and/or differentiation.

The methods of the present invention are useful in preventing, inhibiting or delaying the progressive loss of functional nephron units, and the consequent progressive loss of renal function, which typify chronic renal failure. As such they  
20 are of great value in preventing or delaying the need for chronic dialysis or renal replacement therapy in subjects with chronic renal insufficiency, or reducing the necessary frequency of chronic renal dialysis in subjects with end-stage renal disease. As such, they are useful in prolonging the lives, and in maintaining the quality of life, of subjects at risk of, or already afflicted with, chronic renal failure.

25 In a related aspect, the invention also contemplates conjoint administration of Angiotensin II Receptor Antagonists / Blockers (AIIRAs) with certain protein-based morphogens to subjects in, or at risk of, chronic renal failure, in order to reduce mortality and/or morbidity rates, and to prevent, inhibit, delay or alleviate the progressive loss of renal function which characterizes chronic renal failure.

30 Alternatively, or in addition, conjoint administration of angiotensin II receptor blockers with the morphogens of the present invention can prevent, inhibit or delay

the progressive loss of functional nephron units and the progressive decline in glomerular filtration rate (GFR) which slowly but inevitably leads to the need for renal replacement therapy (i.e., renal transplant or chronic dialysis) or death. In preferred embodiments, the therapeutic agents of the invention are members of the 5 osteogenic protein / bone morphogenetic protein (OP/BMP) family within the TGF- $\beta$  superfamily of proteins, and angiotensin II receptor blockers.

**Brief Description of the Figures**

- Figure 1      The long term streptozotocin induced model of diabetic nephropathy. DM was induced at week 0 and the rats maintained as described in methods. (A) At 16 weeks, kidney weights had increased 1.8 fold in DM compared to normal ( $1.42 \pm 0.02$  versus  $0.81 \pm 0.02$  g,  $p<0.01$ ). (B) GFR also increased 3.2 fold compare to normal ( $1.56 \pm 0.27$  versus  $0.49 \pm 0.04$  ml/mim/100g body wt,  $p<0.01$ ). After 16 weeks of vehicle treatment, kidney weights had not changed significantly (A), but the GFR was decreased 75% to even lower than normal at 32 weeks ( $0.34 \pm 0.02$  versus  $0.55 \pm 0.02$ ) (B).
- Figure 2      Effects of BMP-7 and enalapril treatments on DM induced renal hypertrophy. DM was induced at week 0. Treatment of BMP-7, or enalapril, or vehicle was began at week 16 and finished at week 32. At 16 weeks, kidney weight increased to 1.42 in DM compared to normal. After 16 weeks of vehicle treatment, the kidney weight was still elevated ( $1.44 \pm 0.04$  g). The kidney weights of the BMP-7 and enalapril treated groups were significantly decreased to  $1.10 \pm 0.03$  in BMP-7 high dose group and  $1.09 \pm 0.04$  in enalapril treated group,  $p<0.01$  compared to DM vehicle treated.
- Figure 3      Effects of BMP-7 and enalapril treatments on GFR. In DM rats the GFR was increased 3.2-fold compare to normal at 16 weeks ( $1.56 \pm 0.27$  versus  $0.49 \pm 0.04$  ml/mim/100g body wt,  $p<0.01$ ), but by 32 weeks the GFR was decreased to lower than normal during vehicle treatment ( $0.34 \pm 0.02$  versus  $0.55 \pm 0.02$ ). The BMP-7 and enalapril

treatments restored GFR to normal or slightly above normal. The GFR of the BMP-7 high dose animals were significantly greater than the GFR of DM group (vehicle treated) ( $0.70 \pm 0.08$  versus  $0.34 \pm 0.02$ ,  $p<0.05$ ). There was a dose-dependent organization of the GFR in the BMP-7 treated groups ( $0.59 \pm 0.07$ ,  $0.62 \pm 0.09$ , and  $0.70 \pm 0.08$ , respectively).

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Figure 4 Effects of BMP-7 and enalapril treatments on urine protein excretion in DM. DM was induced at week 0. Treatment of BMP-7, or Enalapril, or vehicle was began at week 16 and finished at week 32. Diabetic rats exhibited a pronounced increase in albumin excretion rate compared with nondiabetic rats at both 16 weeks ( $35.63 \pm 13.35$  versus  $3.76 \pm 0.39$  mg/day) and 32 weeks ( $174.4 \pm 52.50$  versus  $8.24 \pm 1.28$ ,  $p<0.01$ ). This response was markedly reduced by BMP-7 and enalapril treatment ( $p<0.01$ , DM versus BMP10;  $p<0.001$ , DM versus BMP30, BMP100 and Enalapril). There was a dose-dependent inverse order in the levels of urinary protein in the BMP-7 treated groups from low to high dose ( $59.46 \pm 21.84$ ,  $33.02 \pm 9.11$ , and  $14.27 \pm 3.50$ , respectively). The Enalapril treatment group had urinary protein excretion levels similar to the intermediate dose BMP-7 group.

Figure 5 Coronal sections of kidneys stained with periodic acid Schiff to highlight basement membranes and mesangial matrix. Panel A is a section of a kidney from a 16 week normal animal. B is a section of a kidney from a 16 week diabetic animal. Glomerular hypertrophy and early increases in mesangial matrix were present. There was evidence of glomerular (arrowhead) and tubular (arrow) basement membrane thickening. C is a section of a kidney from a 32 week diabetic vehicle-treated animal. One glomerulus is sclerotic and both are hypertrophied. There are segments of collapsed glomerular tuft with sparseness of normal cellular elements. D is a section of a kidney from an animal treated with BMP-7 30  $\mu\text{g}/\text{kg}$  body wt IV twice a

week. E is a section of a kidney from an animal treated with BMP-7 100 µg/kg body wt IV twice a week. F is a section of a kidney from an animal treated with Enalapril 100 mg/l in drinking water. All of the three BMP-7 dosages and enalapril treatment decreased glomerular sclerosis, but the enalapril treatment had more mesangial matrix accumulation.

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Figure 6

Coronal sections of kidneys stained with Gomori's Trichrome for collagen. With this stain, collagen fibrils stain blue, whereas the cells stain red. A and B are sections from kidneys of two normal control animals maintained in the animal facility with the diabetic animals for 32 weeks. C is a section from a diabetic vehicle-treated animal. Arrow shows early interstitial matrix accumulation. The glomeruli are hypertrophic and one has segmental sclerosis. D, E, and F are sections of kidneys from animals treated with BMP-7 10, 30, and 100 µg/kg body wt. IV twice a week, respectively. Kidneys from the 10 µg/kg body wt. dose animals had greater mesangial matrix accumulation than the two higher doses which were very similar to normal except for residual glomerular hypertrophy.

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Effects of BMP-7 and enalapril treatments on glomerular and interstitial areas in DM rats. (A) Glomerular area. Diabetic rats had a significantly larger glomerular area than normal control rats ( $1.28 \pm 0.03$  versus  $0.90 \pm 0.02 \times 104 \mu\text{m}^2$ ,  $p < 0.001$ ). All the treatment groups partially reversed the glomerular hypertrophy ( $p < 0.001$ ). (B) Interstitial area. The cortical interstitial area was increased from  $9.0 \pm 0.6\%$  sections in nondiabetic rat kidneys to  $13.1 \pm 0.7\%$  in the DM rats. The BMP-7 high dose and enalapril treatment significantly decreased interstitial area ( $10.7 \pm 0.3\%$ ,  $p < 0.05$ ;  $10.3 \pm 0.4\%$ ,  $p < 0.01$ , respectively) when compared to vehicle treated DM.

Figure 7

Effects of BMP-7 and enalapril treatments on glomerulosclerosis. Diabetic rats exhibited a significant increase in prevalence of sclerotic glomeruli compared with nondiabetic rats at 32 weeks (10.7

$\pm 4.0\%$  versus  $0.7 \pm 0.2\%$ ,  $p<0.001$ ). This response was markedly reduced by BMP-7 and enalapril treatment. There was a dose-dependent ordering in the BMP-7 treated groups ( $4.1 \pm 1.4\%$ ,  $p<0.05$ ;  $3.5 \pm 0.8\%$ ,  $p<0.01$ ; and  $2.1 \pm 0.3\%$ ,  $p<0.001$ , respectively). The effect of high dose BMP-7 was significantly better than that of Enalapril.

- 5                          Figure 9 Effects of Enalapril and BMP-7 therapies on systolic blood pressure of diabetic rats. Blood pressures were obtained by the artery cuff method. By 16 weeks the diabetic animals were significantly hypertensive. The hypertension was stable in vehicle treated rats until week 28 when systolic blood pressures began to increase again. Over two months Enalapril therapy restored blood pressure to normal. BMP-7 therapy did not affect blood pressure until the last four weeks of therapy.
- 10                         Figure 10 Loss of BMP-7 renal expression in diabetes and restoration with therapy. (A) The 32-week vehicle treated diabetic kidneys had complete loss of BMP-7 message. Both BMP-7 and Enalapril therapy restored the normal distribution of BMP-7 expression. The levels of BMP-7 message in the BMP-7 and Enalapril treated kidneys appeared to be higher than the normal animals. (B) Glomerular expression of BMP-7 during BMP-7 therapy. Bright and dark field sections demonstrate significant BMP-7 expression in a glomerulus of a BMP-7 treated animal. These results were consistent in three separate experiments with different kidneys.
- 15                         Figure 11 Induction of Wnt 4 expression by diabetes and effects of Enalapril and BMP-7 therapy. Wnt4 was not significantly expressed in normal kidneys. However, there was generalized renal expression of Wnt4 in vehicle treated diabetic rats. The expression was both in tubular epithelial cells and the glomeruli. BMP-7 therapy and Enalapril therapy had no effect on Wnt4 expression. The results were consistent in three separate experiments involving different kidneys.
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- Figure 12 Schematic diagram of the five-sixths nephrectomy (5/6 NPX) Chronic Renal Failure (CRF) injury model.
- Figure 13 OP-1 did not dramatically lower the blood pressure level in the 5/6 nephrectomy model of Chronic Renal Failure.
- 5 Figure 14 OP-1 significantly reduced the proteinuria level in the 5/6 nephrectomy model of Chronic Renal Failure.
- Figure 15 In animals conjointly administered with morphogen (OP-1) and ACE inhibitor (enalapril), there is no additional benefit in reducing the blood pressure of nephrectomized animals to normal level as compared to animals treated by the ACE inhibitor (enalapril) alone.
- 10 Figure 16 Conjoint administration of morphogen (OP-1) and ACEI (enalapril) is more effective in reducing the proteinuria level in nephrectomized animals than ACEI treatment alone.
- Figure 17 Schematic diagram of the Unilateral Ureteral Obstruction (UUO) Renal Fibrosis Model.
- 15 Figure 18 Morphogen OP-1 / BMP-7 Inhibits renal fibrosis in the Unilateral Ureteral Obstruction model.
- Figure 19 The mechanism of morphogen (OP-1)- induced renal protection is associated with prevention of tubular atrophy, an effect not shared with ACEI enalapril.
- 20 Figure 20 Both morphogen and ACEI improves renal function as measured by GFR. However, morphogen OP-1 is more efficacious than ACEI enalapril in improving the glomerular filtration rate as evidenced by the inulin clearance rate in the Unilateral Ureteral Obstruction model.
- 25 Figure 21 Morphogen (OP-1), but not ACE inhibitor, significantly reduced the loss of medullary tissue in the kidney in the Unilateral Ureteral Obstruction model.
- Figure 22 Schematic diagram of Streptozotocin-induced rat model of diabetic nephropathy.

- Figure 23 Morphogen (OP-1), but not ACEI enalapril, significantly increased the glomerular filtration rate (GFR) in the Streptozotocin-induced rat model of diabetic nephropathy.
- 5 Figure 24 Administration of morphogen (OP-1) alone, or ACEI enalapril alone, or conjoint administration of morphogen and ACEI significantly reduced the proteinuria level in the Streptozotocin-induced rat model of diabetic nephropathy.
- Figure 25 Schematic diagram of the Alloxan-induced rat model of diabetic nephropathy.
- 10 Figure 26 Morphogen OP-1 dramatically reduces the serum creatinine level, while ACEI elalapril reduces the serum creatinine level at a lesser degree in the Alloxan-induced rat model of diabetic nephropathy.
- Figure 27 Morphogen OP-1 and ACEI enalapril both reduce the preteinuria level. However, conjoint administration of morphogen and ACEI synergistically reduce the preteinuria level in the Alloxan-induced rat model of diabetic nephropathy.
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- Figure 28 Comparison of percent amino acid sequence homology / similarity and percent identity within the C-terminal seven cysteine skeletons of various representative members of the TGF- $\beta$  superfamily proteins, using OP-1 as the reference sequence. The percent homologies recited in the figure are based on aligning the sequences using the 20 MegaAlign Program (DNAstar, Inc.).

### **Detailed Description of the Invention**

I. Overview

25 The present invention depends, in part, upon the surprising discovery that conjoint administration of ACE inhibitors with certain protein-based morphogens to subjects in, or at risk of, chronic renal failure, can reduce mortality and/or morbidity rates, and prevent, inhibit, delay or alleviate the progressive loss of renal function which characterizes chronic renal failure. Alternatively, or in addition, conjoint

administration of ACE inhibitors with the morphogens of the present invention can prevent, inhibit or delay the progressive loss of functional nephron units and the progressive decline in glomerular filtration rate (GFR) which slowly but inevitably leads to the need for renal replacement therapy (i.e., renal transplant or chronic dialysis) or death. In preferred embodiments, the therapeutic agents of the invention are members of the osteogenic protein / bone morphogenetic protein (OP/BMP) family within the TGF- $\beta$  superfamily of proteins, and inhibitors of the ACE family of proteins.

The present invention also contemplates conjoint administration of Angiotensin II Receptor Antagonists / Blockers (AIIRAs) with certain protein-based morphogens to subjects in, or at risk of, chronic renal failure, in order to reduce mortality and/or morbidity rates, and to prevent, inhibit, delay or alleviate the progressive loss of renal function which characterizes chronic renal failure. Alternatively, or in addition, conjoint administration of angiotensin II receptor blockers with the morphogens of the present invention can prevent, inhibit or delay the progressive loss of functional nephron units and the progressive decline in glomerular filtration rate (GFR) which slowly but inevitably leads to the need for renal replacement therapy (i.e., renal transplant or chronic dialysis) or death. In preferred embodiments, the therapeutic agents of the invention are members of the osteogenic protein / bone morphogenetic protein (OP/BMP) family within the TGF- $\beta$  superfamily of proteins, and angiotensin II receptor blockers.

## II. Definitions

In order to more clearly and concisely point out the subject matter of the claimed invention, the following definitions are provided for specific terms used in the following written description and appended claims.

OP/BMP morphogen. As used herein, the term "OP/BMP morphogen" means a polypeptide, or a functional variant of a polypeptide, comprising at least the C-terminal six- or seven-cysteine domain of a mammalian protein selected from the group consisting of OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, BMP9, and proteins which exhibit at least 65% or, more preferably, 70%, 75% 80%,

85%, 90%, or 95% amino acid sequence homology, or at least 50%, more preferably 55%, 60%, 70%, 80%, 90%, 99% or more identity, with the amino acid sequence of the seven-cysteine domain of any one of the morphogens described above, such as human OP-1 (SEQ ID NO: 1); and which is (a) capable of inducing chondrogenesis 5 in the Reddi-Sampath ectopic bone assay (Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA) 78: 7599-7603) or a substantially equivalent assay, (b) capable of significantly preventing, inhibiting, delaying or alleviating the progressive loss of renal function in a standard animal model of chronic renal failure, or (c) capable of causing a clinically significant improvement in a standard marker of renal function 10 when administered to a mammal in, or at risk of, chronic renal failure.

As used herein, "amino acid sequence homology" or a percentage "homology" between two amino acid sequences is understood herein to include both amino acid sequence identity and conserved substitution. Thus, as used herein, a percentage "homology" between two amino acid sequences indicates the percentage 15 of amino acid residues which are identical or are conserved substitution between the sequences. "Conservative substitutions" of amino acids fulfill the criteria defined for an "accepted point mutation" in Dayhoff et al. (1978), Atlas of Protein Sequence and Structure Vol. 5 (Suppl. 3), pp. 354-352, Natl. Biomed. Res. Found., Washington, D.C. Thus, "conservative substitutions" are residues that are physically or 20 functionally similar to the corresponding reference residues, having similar size, shape, electric charge, and/or chemical properties such as the ability to form covalent or hydrogen bonds, or the like. Examples of preferred conservative substitutions include the substitution of one amino acid for another with similar characteristics, e.g., substitutions within the following groups: (a) Ser, Thr, Pro, Ala, 25 Gly; (b) Asn, Asp, Glu, Gln; (c) His, Arg, Lys; (d) Met, Ile, Leu, Val; (e) Phe, Tyr, Trp. In a most preferred embodiment, conservative substitutions include the substitution of one amino acid for another within the following groups: (a) glycine, alanine; (b) valine, isoleucine, leucine; (c) aspartic acid, glutamic acid; (d) asparagine, glutamine; (e) serine, threonine; (f) lysine, arginine, histidine; and (g) 30 phenylalanine, tyrosine. See Figure 84 of Dayhoff et al. (1978), Atlas of Protein Sequence and Structure Vol. 5 (Suppl. 3), pp. 354-352, Natl. Biomed. Res. Found., Washington, D.C. The term "conservative substitution" or "conservative variation"

also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid in a given polypeptide chain, provided that the resulting substituted polypeptide chain also has therapeutic efficacy in the present invention.

As used herein, a therapeutic agent (morphogen and/or ACEI) of the  
5 invention is said to have "therapeutic efficacy," and an amount of the agent is said to be "therapeutically effective," if administration of that amount of the agent is sufficient to cause a clinically significant improvement in a standard marker of renal function when administered to a mammalian subject (e.g., a human patient) in, or at risk of, chronic renal failure. Such markers of renal function are well known in the  
10 medical literature and include, without being limited to, rates of increase in BUN levels, rates of increase in serum creatinine, static measurements of BUN, static measurements of serum creatinine, glomerular filtration rates (GFR), ratios of BUN/creatinine, serum concentrations of sodium ( $\text{Na}^+$ ), urine/plasma ratios for creatinine, urine/plasma ratios for urea, urine osmolarity, daily urine output, and the  
15 like (see, for example, Brenner and Lazarus(1994), in Harrison's Principles of internal Medicine, 13th edition, Isselbacher et al., eds., McGraw Hill Text, New York; Luke and Strom (1994), in Internal Medicine, 4th Edition, J.H. Stein, ed., Mosby-Year Book, Inc. St. Louis).

As used herein, "conjoint administration" means administration of two or  
20 more agents to a subject of interest as part of a single therapeutic regimen. The administration(s) can be either simultaneous or sequential, i.e., administering one agent followed by administering of a second (and/or a third one, etc.) at a later time, as long as the agents administered co-exist in the subject being treated, or at least one agent will have the opportunity to act upon the same target tissues of other  
25 agents while said target tissues are still under the influence of said other agents. In a preferred embodiment, agents to be administered can be included in a single pharmaceutical composition and administered together. In another preferred embodiment, the agents are administered simultaneously, including through separate routes. In yet another preferred embodiment, one or more agents are administered  
30 continuously, while other agents are administered only at predetermined intervals (such as a single large dosage, or twice a week at smaller dosages, etc.). For

example, the morphogens can be administered three times a week through direct injection, while the ACE inhibitors can be continuously released by an implant.

The route of administration can be the same or different, depending on needs or suitable methods of administration for each agent. Any suitable route of  
5 administration may be employed for providing the patient with effective dosages of an ACEI and a morphogen. For example, oral, rectal, parenteral, transdermal, subcutaneous, intramuscular, inhalation and the like may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, patches and the like. In certain embodiments, the morphogen can be administered via direct  
10 injection, while the ACE inhibitors can be administered through drinking water.

As used herein, "prevent" or "prevention" means reducing the probability / risk of developing a condition in a subject (cell, tissue, organ, or organism, etc.), or delaying the onset of a condition in the subject, or to lessening the severity of one or more symptoms of a condition that may develop in the subject, or any combination  
15 thereof.

Filtration. The kidneys' main function is to remove toxins (uremic wastes) that accumulate in the blood as a result of the body's metabolism. The body continuously uses digested elements from foods and stored nutrients to perform normal bodily functions. The by-products of nutrient metabolism and cell function  
20 are filtered from the blood by the kidneys, which excrete (discharge) wastes as urine. Every day, approximately 200 liters of blood flow to the kidneys where 2 liters of waste are filtered out.

BUN and Creatinine. The concentration in the blood (blood level) of blood urea nitrogen (BUN), known as urea, and creatinine (Cr) can be measured by routine  
25 laboratory tests. BUN and creatinine levels indicate the general function of the kidneys. BUN is a metabolic by-product of protein-rich food such as meat, poultry, and certain vegetables. BUN is filtered out of the blood by the kidneys and excreted in the urine. Creatinine is continuously generated by normal cell metabolism within the muscles. Creatinine is also filtered out of the blood by the kidneys and excreted  
30 in the urine.

The amounts of BUN and creatinine in the blood are equal to the amount excreted by the kidneys. The blood levels of BUN and Cr remain unchanged unless there is sudden deterioration of renal (i.e., kidney) function. If the kidneys are suddenly unable to function, BUN and Cr increase daily. This condition is known as 5 *acute renal failure*. *Chronic renal failure* is a condition distinguished by a gradual increase in BUN and Cr over a long period of time.

- Measurement of Kidney Function. When renal function decreases, blood levels of Cr and BUN increase because the kidneys are unable to clean the blood effectively. Factors not related to the kidneys also impact BUN and Cr levels.
- 10 Creatinine, in particular, is affected by age, sex, weight, and muscle mass.

Renal function is measured to evaluate the rate at which both kidneys are able to clean the blood. To measure renal function, a 24-hour urine sample must be collected. It is of importance that the 24-hour sample is complete (i.e., no urine is missing), so that true renal function will not be underestimated.

15 The amount of Cr in the urine sample is compared to the blood level of Cr. This figure is known as creatinine clearance (CrCl), the rate at which both kidneys clean the blood. The normal CrCl is about 90 to 130 milliliters per minute (mL/min). Many people gradually lose renal function as they age. Alternative renal function measurements rely on tables or formulas that take into consideration age, body 20 weight, sex, and blood creatinine.

Some health care facilities in the United States offer the Gofil-125 assay to evaluate renal function. Sodium iothalamate I-125 (a radiopharmaceutical) is injected into the skin, and blood and urine samples are obtained to determine renal function. The test is easy to perform, is more sensitive than blood creatinine 25 measurements, and provides results within 2 to 3 hours. Measurements of renal function determine the severity of kidney impairment. It is important to monitor renal function over time to document the rate of deterioration or improvement with treatment.

Glomerular Filtration Rate (GFR). The "glomerular filtration rate" or "GFR" 30 is proportional to the rate of clearance into urine of a plasma-borne substance which is not bound by serum proteins, is freely filtered across glomeruli, and is neither

secreted nor reabsorbed by the renal tubules. Thus, as used herein, GFR preferably is defined by the following equation.

$$GFR = U_{conc} \times V / P_{conc}$$

where  $U_{conc}$  is the urine concentration of the marker,  $P_{conc}$  is the plasma

- 5 concentration of the marker, and  $V$  is the urine flow rate in ml/min. Optionally, GFR is corrected for body surface area. Thus, the GFR values used herein may be regarded as being in units of ml/min/1.73m<sup>2</sup>.

The preferred measure of GFR is the clearance of insulin but, because of the difficulty of measuring the concentrations of this substance, the clearance of  
10 creatinine is typically used in clinical settings. For example, for an average size, healthy human male (70 kg, 20-40 yrs), a typical GFR measured by creatinine clearance is expected to be approximately 125 ml/min with plasma concentrations of creatinine of 0.7-1.5 mg/dL. For a comparable, average size woman, a typical GFR measured by creatinine clearance is expected to be approximately 115 ml/min with creatinine levels of 0.5-1.3 mg/dL. During times of good health, human GFR values are relatively stable until about age 40, when GFR typically begins to decrease with age. For subjects surviving to age 85-90, GFR may be reduced to 50% of the comparable values at 40.

Expected Glomerular Filtration Rate (GFR<sub>exp</sub>). An estimate of the "expected GFR" or "GFR<sub>exp</sub>" may be provided based upon considerations of a subject's age, weight, sex, body surface area, and degree of musculature, and the plasma concentration of some marker compound (e.g., creatinine) as determined by a blood test. Thus, as an example, an expected GFR or GFR<sub>exp</sub> maybe estimated as:

$$GFR_{exp} = (140 - \text{age}) \times \text{weight (kg)} / [72 \times P_{conc} (\text{mg/dl})]$$

25 This estimate does not take into consideration such factors as surface area, degree of musculature, or percentage body fat. Nonetheless, using plasma creatinine levels as the marker, this formula has been employed for human males as an inexpensive means of estimating GFR. Because creatinine is produced by striated muscle, the expected GFR or GFR<sub>exp</sub> of human female subjects is estimated by the

same equation multiplied by 0.85 to account for expected differences in muscle mass. (See Lemann, et al. (1990) Am. J. Kidney Dis. 16(3): 236).

Broad Cast. Microscopic examination of urinary sediment for the presence of formed elements is a standard procedure in urinalysis. Amongst the formed elements which may be present in urine are cylindrical masses of agglutinated materials that typically represent a mold or "cast" of the lumen of a distal convoluted tubule or collecting tubule. In healthy human subjects, such casts typically have a diameter of 15-25  $\mu\text{m}$ . In subjects with chronic renal failure, however, hypertrophy of the tubules may result in the presence of "broad casts" or "renal failure casts" which are 2-6 times the diameter of normal casts and often have a homogeneous waxy appearance. Thus, as used herein, a "broad cast" means a urinary sediment cast having a diameter of 2-6 times normal, or about 30-150  $\mu\text{m}$  for human casts.

Chronic. As used herein with respect to clinical indications such as urinary casts, measured GFR, or other markers of renal function, "chronic" means persisting for a period of at least three, and more preferably, at least six months. Thus, for example, a subject with a measured GFR chronically below 50% of  $\text{GFR}_{\text{exp}}$  is a subject in which the GFR has been measured and found to be below 50% of  $\text{GFR}_{\text{exp}}$  in at least two measurements separated by at least three, and more preferably, by at least six months, and for which there is no medically sound reason to believe that GFR was substantially (e.g., 10%) higher during the intervening period.

Subjects in, or at risk of, chronic renal failure. As used herein, a subject is said to be in, or at risk of chronic renal failure, or at risk of the need for renal replacement therapy, if the subject is reasonably expected to suffer a progressive loss of renal function associated with progressive loss of functioning nephron units. Whether a particular subject is in, or at risk of, chronic renal failure is a determination which may routinely be made by one of ordinary skill in the relevant medical or veterinary art. Subjects in, or at risk of, chronic renal failure, or at risk of the need for renal replacement therapy, include but are not limited to the following: subjects which may be regarded as afflicted with chronic renal failure, end-stage renal disease, chronic diabetic nephropathy, hypertensive nephrosclerosis, chronic glomerulonephritis, hereditary nephritis, and/or renal dysplasia; subjects having a

biopsy indicating glomerular hypertrophy, tubular hypertrophy, chronic glomerulosclerosis, and/or chronic tubulointerstitial sclerosis; subjects having an ultrasound, NMR, CAT scan, or other non-invasive examination indicating renal fibrosis; subjects having an unusual number of broad casts present in urinary sediment; subjects having agar which is chronically less than about 50%, and more particularly less than about 40%, 30% or 20%, of the expected GFR for the subject; human male subjects weighing at least about 50 kg and having a GFR which is chronically less than about 50 ml/min, and more particularly less than about 40 ml/min, 30 ml/min or 20 ml/min; human female subjects weighing at least about 40 kg and having a GFR which is chronically less than about 40 ml/min, and more particularly less than about 30 ml/min, 20 ml/min or 10 ml/min; subjects possessing a number of functional nephron units which is less than about 50%, and more particularly less than about 40%, 30% or 20%, of the number of functional nephron units possessed by a healthy but otherwise similar subject; subjects which have a single kidney; and subjects which are kidney transplant recipients.

### **III. Description of the Preferred Embodiments**

#### *A. Therapeutic Agents*

The morphogens of the present invention are naturally occurring proteins, or functional variants of naturally occurring proteins, in the osteogenic protein / bone morphogenetic protein (OP/BMP) family within the TGF- $\beta$  superfamily of proteins.

The ACE inhibitors of the invention are generally small organic molecules. "Small molecule" as used herein, is meant to refer to a composition, which has a molecular weight of less than about 5 kD and most preferably less than about 2.5 kD. Small molecules can be nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic (carbon containing) or inorganic molecules.

##### (i) OP/BMP family of morphogens

The "OP/BMP family" of proteins forms a distinct subgroup, within the loose evolutionary grouping of sequence-related proteins known as the TGF- $\beta$  superfamily. Members of this protein family comprise secreted polypeptides that share common structural features, and that are similarly processed from a pro-

protein to yield a carboxy-terminal mature protein. This family of proteins is also referred to as morphogens. As noted above, a protein is morphogenic as defined herein if it induces the developmental cascade of cellular and molecular events that culminate in the formation of new, organ-specific tissue.

5 It has been discovered that morphogens enhance survival of neurons and maintain neural pathways. As described herein, morphogens are capable of enhancing survival of neurons, stimulating neuronal CAM expression, maintaining the phenotypic expression of differentiated neurons, inducing the redifferentiation of transformed cells of neural origin, and stimulating axonal growth over breaks in  
10 neural processes, particularly large gaps in axons. Morphogens also protect against tissue destruction associated with immunologically related nerve tissue damage. In addition, morphogens may be used as part of a method for monitoring the viability of nerve tissue in a mammal.

In a preferred embodiment, a morphogen is a dimeric protein, each  
15 polypeptide component of which has a sequence that corresponds to, or is functionally equivalent to, at least the conserved C-terminal six or seven cysteine skeleton of human OP-1, included in SEQ ID NO: 2, and/or which shares 70% amino acid sequence homology or 50% identity with OP-1 in this region. The morphogens are generally competent to induce a cascade of events including the  
20 following, in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells. Under appropriate conditions, morphogens are also competent to induce redifferentiation of cells that have undergone abnormal differentiation.  
25 Details of how the morphogens useful in this invention were identified, as well as a description on how to make, use and test them for morphogenic activity are disclosed in numerous publications, including U.S. Patent Nos. 5,011,691 and 5,266,683, and the international patent application publications WO 92/15323; WO 93/04692; and WO 94/03200, each of which are incorporated by reference herein.  
30 As disclosed therein, the morphogens can be purified from naturally sourced material or recombinantly produced from prokaryotic or eukaryotic host cells, using

the genetic sequences disclosed therein. Alternatively, novel morphogenic sequences can be identified following the procedures disclosed therein.

The naturally occurring morphogens share substantial amino acid sequence homology in their C-terminal sequences (sharing, e.g., a six or seven cysteine 5 skeleton sequence). Typically, a naturally occurring morphogen is translated as a precursor, having an N-terminal signal peptide sequence, typically less than about 35 residues in length, followed by a "pro" domain that is cleaved to yield the mature polypeptide, which includes the biologically active C-terminal skeleton sequence. The signal peptide is cleaved rapidly upon translation, at a cleavage site that can be 10 predicted in a given sequence using the method of Von Heijne, *Nucleic Acids Research* 14: 4683-4691 (1986). The "pro" domain is variable both in sequence and in length, ranging from approximately 200 to over 400 residues. The pro domain is cleaved to yield the "mature" C-terminal domain of approximately 115-180 residues, which includes the conserved six- or seven-cysteine C-terminal domain of 97-106 15 residues. The pro polypeptide typically is about three times larger than the fully processed, mature C-terminal polypeptide. Under native conditions, the protein is secreted as a mature dimer and the cleaved pro polypeptide is thought to remain associated therewith to form a protein complex, presumably to improve the solubility of the mature dimeric protein. The complexed form of a morphogen is 20 generally observed to be more soluble than the mature form under physiological conditions.

As used herein, the "pro form" of an OP/BMP family member refers to a protein comprising a folded pair of polypeptides, each comprising a pro domain in either covalent or noncovalent association with the mature domains of the OP/BMP 25 polypeptide. The pro form appears to be the primary form secreted from cultured mammalian cells. The "mature form" of the protein refers to mature C-terminal domain which is not associated, either covalently or noncovalently, with the pro domain. Any preparation of OP-1 is considered to contain mature form when the amount of pro domain in the preparation is no more than 5% of the amount of 30 "mature" C-terminal domain.

Natural-sourced morphogenic protein in its mature, native form, is typically a glycosylated dimer, having an apparent molecular weight of about 30-36 kDa as determined by SDS-PAGE. When reduced, the 30 kDa protein gives rise to two glycosylated polypeptide subunits having apparent molecular weights in the range of 5 about 16 kDa and about 18 kDa. The unglycosylated dimeric protein, which also has morphogenic activity, typically has an apparent molecular weight in the range of about 27 kDa. When reduced, the 27 kDa protein gives rise to two unglycosylated polypeptides having molecular weights typically in the range of about 14 kDa to about 16 kDa.

10 OP/BMP family members useful herein include any of the known naturally occurring native proteins including allelic, phylogenetic counterpart and other variants thereof, whether naturally sourced or biosynthetically produced (e.g., including "muteins" or "mutant proteins"), as well as new, active members of the OP/BMP family of proteins. Particularly useful sequences include those comprising 15 the C-terminal seven cysteine domains of mammalian, preferably human, OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, BMP8 and BMP9. Other proteins useful in the practice of the invention include active forms of DPP, Vg1, Vgr-1, 60A, GDF-1, GDF-3, GDF-5, GDF-6, GDF-7, BMP10, BMP11; BMP13, BMP15, UNIVIN, NODAL, SCREW, ADMP or NURAL and amino acid sequence variants 20 thereof. In one preferred embodiment, the morphogens of the invention are selected from any one of OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, and BMP9.

In preferred embodiments, each of the polypeptide subunits of a dimeric morphogenic protein as defined herein comprises an amino acid sequence sharing a 25 defined relationship with an amino acid sequence of a reference morphogen. In one embodiment, preferred morphogenic polypeptide chains share a defined relationship with a sequence present in morphogenically active full-length human OP-1, SEQ ID NO: 3. However, any one or more of the naturally occurring or biosynthetic morphogenic proteins disclosed herein similarly could be used as a reference 30 sequence. Preferred morphogenic polypeptide chains share a defined relationship with at least the C-terminal six cysteine skeleton of human OP-1, residues 335-431

of SEQ ID NO: 3 (or residues 38-139 of SEQ ID NO: 1). Preferably, morphogenic proteins share a defined relationship with at least the C-terminal seven cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 3 (or residues 38-139 of SEQ ID NO: 1).

- 5        Functionally equivalent sequences include functionally equivalent arrangements of cysteine residues disposed within the reference sequence, including amino acid insertions or deletions which alter the linear arrangement of these cysteines, but do not materially impair their relationship in the folded structure of the dimeric morphogen protein, including their ability to form such intra- or inter-  
10      chain disulfide bonds as may be necessary for morphogenic activity. For example naturally occurring morphogens have been described in which at least one internal deletion (of one residue; BMP2) or insertion (of four residues; GDF-1) is present but does not abrogate biological activity. Functionally equivalent sequences further include those wherein one or more amino acid residues differ from the  
15      corresponding residue of a reference sequence, *e.g.*, the C-terminal seven cysteine skeleton of human OP-1, provided that this difference does not destroy tissue morphogenic activity. Accordingly, conservative substitutions of corresponding amino acids in the reference sequence are preferred. Amino acid residues that are "conservative substitutions" for corresponding residues in a reference sequence are  
20      those that are physically or functionally similar to the corresponding reference residues, *e.g.*, that have similar size, shape, electric charge, chemical properties including the ability to form covalent or hydrogen bonds, or the like. Particularly preferred conservative substitutions are those fulfilling the criteria defined for an accepted point mutation in Dayhoff, *et al.*, 5 ATLAS OF PROTEIN SEQUENCE AND  
25      STRUCTURE, Suppl. 3, ch. 22 pp. 354-352 (1978), Natl. Biomed. Res. Found., Washington, D.C. (*supra*), the teachings of which are incorporated by reference herein. The term "conservative substitution" also includes the use of a synthetic or derivatized amino acid in place of the corresponding natural parent amino acid, provided that antibodies raised to the resulting variant polypeptide also immunoreact  
30      with the corresponding naturally sourced morphogen polypeptide.

Among the morphogens useful in this invention are proteins originally identified as osteogenic proteins, such as the OP-1, OP-2 and CBMP2 proteins, as well as amino acid sequence-related proteins such as DPP (from *Drosophila*), Vgl (from *Xenopus*), Vgr-1 (from mouse, see U.S. 5,011,691 to Oppermann et al.), GDF-5 1 (from mouse, see Lee (1991) *PNAS* 88: 4250-4254), all of which are presented in Table II, and the recently identified 60A protein (from *Drosophila*, see Wharton et al. (1991) *PNAS* 88: 9214-9218). As mentioned before, the members of this family, which include members of the TGF- $\beta$  super-family of proteins, share substantial amino acid sequence homology in their C-terminal regions. The proteins are 10 translated as a precursor, having an N-terminal signal peptide sequence, typically less than about 30 residues, followed by a "pro" domain that is cleaved to yield the mature sequence. The signal peptide is cleaved rapidly upon translation, at a cleavage site that can be predicted in a given sequence using the method of Von Heijne ((1986) *Nucleic Acids Research* 14:4683-4691.)

15 Table I below summarizes various naturally occurring members of the OP/BMP family identified to date, including their nomenclature as used herein, their Sequence listing references, and publication sources for the amino acid sequences for the full length proteins not included in the Sequence listing. Each of the generic terms set forth in Table I is intended and should be understood to embrace the 20 therapeutic effective proteins expressed from nucleic acids encoding the identified sequence mentioned below and set forth in the Sequence listing, or an active fragment or precursor thereof, or a functional equivalent thereof such as a naturally occurring or biosynthetic variant. Naturally occurring variants include allelic variant forms isolated from other individuals of a single biological species, as well as 25 species variants (homologous) isolated from phylogenetically distinct biological species.

Table I            Exemplary Morphogens

30            "OP-1"            Refers generically to the group of morphogenically active proteins expressed from part or all of a DNA sequence encoding OP-1 protein, including allelic and species variants thereof, e.g., human OP-1 ("hOP-1", SEQ ID NO: 1, mature protein amino acid sequence), or mouse OP-1 ("mOP-1", SEQ ID NO: 4, mature protein amino acid sequence.) The conserved seven cysteine skeleton is defined by

5 residues 38 to 139 of SEQ ID NOs: 1 and 4. The cDNA sequences and the amino acids encoding the full length proteins are provided in SEQ ID NOs: 17 and 3 (hOP-1) and SEQ ID NOs: 18 and 19 (mOP-1). The mature proteins are defined by residues 293-431 (hOP-1) and 292-430 (mOP-1). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 30-292 (hOP-1) and residues 30-291 (mOP-1).

10 "OP-2" refers generically to the group of active proteins expressed from part or all of a DNA sequence encoding OP-2 protein, including allelic and species variants thereof, e.g., human OP-2 ("hOP-2", SEQ ID NO: 5, mature protein amino acid sequence) or mouse OP-2 ("mOP-2", SEQ ID NO: 6, mature protein amino acid sequence). The conserved seven cysteine skeleton is defined by residues 38 to 139 of SEQ ID NOs: 5 and 6. The cDNA sequences and the amino acids encoding the full length proteins are provided in SEQ ID NOs: 20 and 21 (hOP-2) and SEQ ID NOs: 22 and 23 (mOP-2). The mature proteins are defined essentially by residues 264-402 (hOP-2) and 261-399 (mOP-2). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins likely are defined essentially by residues 18-263 (hOP-2) and residues 18-260 (mOP-2). Another cleavage site also occurs 21 residues upstream for both OP-2 proteins.

15 "CBMP2" refers generically to the morphogenically active proteins expressed from a DNA sequence encoding the CBMP2 proteins, including allelic and species variants thereof, e.g., human CBMP2A ("CBMP2A(fx)", SEQ ID NO: 10) or human CBMP2B DNA ("CBMP2B(fx)", SEQ ID NO: 11). The amino acid sequence for the full length proteins, referred to in the literature as BMP2A and BMP2B, or BMP2 and BMP4, appear in Wozney, et al. (1988) Science 242:1528-1534, the content of which is incorporated by reference herein. The pro-domain for BMP2 (BMP2A) likely includes residues 25-248 or 25-282; the mature protein, residues 249-396 or 283-396. The pro-domain for BMP4 (BMP2B) likely includes residues 25-256 or 25-292; the mature protein, residues 257-408 or 293-408.

20 "DPP(fx)" refers to protein sequences encoded by the *Drosophila* DPP gene (DPP protein, see SEQ ID NO: 7) and defining the conserved seven cysteine skeleton. The amino acid sequence for the full length protein appears in Padgett, et al (1987) Nature 325: 81-84, the content of which is incorporated by reference herein. The pro-domain likely extends from the signal peptide cleavage site to residue 456; mature protein likely is defined by residues 457-588. The sequence of DPP(fx) is shown in Table II.

25 "Vgl(fx)" refers to protein sequences encoded by the *Xenopus* Vgl gene (Vgl protein, see SEQ ID NO: 8) and defining the conserved seven cysteine

- 5 skeleton. The amino acid sequence for the full length protein appears in Weeks (1987) Cell 51: 861-867, the content of which is incorporated by reference herein. The pro-domain likely extends from the signal peptide cleavage site to residue 246; the mature protein likely is defined by residues 247-360. The sequence of Vg1(fx) is shown in Table II.
- 10 ""Vgr-1(fx)" refers to protein sequences encoded by the murine vgr-1 gene (Vgr-1 protein, see SEQ ID NO: 9) and defining the conserved seven cysteine skeleton. The amino acid sequence for the full length protein appears in Lyons, et al., (1989) PNAS 86: 4554-4558, the content of which is incorporated by reference herein. The pro-domain likely extends from the signal peptide cleavage site to residue 299; the mature protein likely is defined by residues 300-438. The sequence of Vgr-1(fx) is shown in Table II.
- 15 "GDF-1(fx)" refers to protein sequences encoded by the human GDF-1 gene (GDF-1 protein, see SEQ ID NO: 13) and defining the conserved seven cysteine skeleton. The amino acid sequence for the full length protein is provided in SEQ ID NO: 13. The pro-domain likely extends from the signal peptide cleavage site to residue 214; the mature protein likely is defined by residues 215-372. The sequence of GDF-1(fx) is shown in Table II.
- 20 25 "60A" refers generically to the morphogenically active proteins expressed from part or all of a DNA sequence (from the *Drosophila* 60A gene) encoding the 60A proteins (see SEQ ID NO: 14). "60A(fx)" refers to the protein sequences defining the conserved seven cysteine skeleton (residues 354 to 455 of SEQ ID NO: 14). The pro-domain likely extends from the signal peptide cleavage site to residue 324; the mature protein likely is defined by residues 325-455. The sequence of 60A(fx) is shown in Table II.
- 30 35 "BMP3(fx)" refers to protein sequences encoded by the human BMP3 gene (BMP3 protein, see SEQ ID NO: 12) and defining the conserved seven cysteine skeleton. The amino acid sequence for the full length protein appears in Wozney et al. (1988) Science 242: 1528-1534, the content of which is incorporated by reference herein. The pro-domain likely extends from the signal peptide cleavage site to residue 290; the mature protein likely is defined by residues 291-472. The sequence of BMP3(fx) is shown in Table II.
- 40 45 "BMP5(fx)" refers to protein sequences encoded by the human BMP5 gene (BMP5 protein, see SEQ ID NO: 15) and defining the conserved seven cysteine skeleton. The amino acid sequence for the full length protein appears in Celeste, et al. (1991) PNAS 87: 9843-9847, the content of which is incorporated by reference herein. The pro-domain likely extends from the signal peptide cleavage site to residue 316; the

mature protein likely is defined by residues 317-454. The sequence of BMP5(fx) is shown in Table II.

5 "BMP6(fx)" refers to protein sequences encoded by the human BMP6 gene (BMP6 protein, see SEQ ID NO: 16) and defining the conserved seven cysteine skeleton. The amino acid sequence for the full length protein appears in Celeste, et al. (1990) PNAS 87: 9843-9847, the content of which is incorporated by reference herein. The pro-domain likely includes extends from the signal peptide cleavage site to residue 374; 10 the mature sequence likely includes residues 375-513. The sequence of BMP6(fx) is shown in Table II.

15 The OP-2 proteins have an "additional" cysteine residue in this region (e.g., see residue 41 of SEQ ID NOS: 21 and 23), in addition to the conserved cysteine skeleton in common with the other proteins in this family. The GDF-1 protein has a four amino acid insert within the conserved skeleton (compare SEQ ID NO: 19 with SEQ ID NO: 13) but this insert likely does not interfere with the relationship of the cysteines in the folded structure. In addition, the CBMP2 proteins are missing one 20 amino acid residue within the cysteine skeleton.

The morphogens are inactive when reduced, but are active as oxidized homodimers and when oxidized in combination with other morphogens of this invention (e.g., as heterodimers). Thus, as defined herein, a morphogen is a dimeric protein comprising a pair of polypeptide chains, wherein each polypeptide chain 25 comprises at least the C-terminal six cysteine skeleton defined by residues 43-139 of SEQ ID NO: 1, including functionally equivalent arrangements of these cysteines (e.g., amino acid insertions or deletions which alter the linear arrangement of the cysteines in the sequence but not their relationship in the folded structure), such that, when the polypeptide chains are folded, the dimeric protein species comprising the 30 pair of polypeptide chains has the appropriate three-dimensional structure, including the appropriate intra-or inter-chain disulfide bonds such that the protein is capable of acting as a morphogen as defined herein. Specifically, the morphogens generally are capable of all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the 35 differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells, including

the "redifferentiation" of transformed cells. In addition, it is also anticipated that these morphogens are capable of inducing redifferentiation of committed cells under appropriate environmental conditions.

The following publications disclose published morphogen polypeptide sequences, as well as relevant chemical and physical properties, of naturally occurring and/or synthetic morphogens: OP-1 and OP-2: U.S. 5,011,691, U.S. 5,266,683, Ozkaynak, *et al.*, *EMBO J.* 9: 2085-2093 (1990); OP-3: WO 94/10203 (PCT US93/10520); BMP-2, BMP-3, and BMP-4: WO 88/00205, Wozney, *et al.*, *Science* 242: 1528-1534 (1988); BMP-5 and BMP-6: Celeste, *et al.*, *PNAS* 87: 9843-9847 (1991); Vgr1: Lyons, *et al.*, *PNAS* 86: 4554-4558 (1989); DPP: Padgett, *et al.*, *Nature* 325: 81-84 (1987); Vg-1: Weeks *Cell* 51: 861-867 (1987); BMP-9: WO 95/33830 (PCT/US95/07084); BMP-10: WO 94/26893 (PCT/US94/05290); BMP-11: WO 94/26892 (PCT/US94/05288); BMP-12: WO 95/16035 (PCT/US94/14030); BMP-13: WO 95/16035 (PCT/US94/14030); GDF-1: WO 92/00382 (PCT/US91/04096) and Lee, *et al.*, *PNAS* 88:4250-4254 (1991); GDF-8: WO 94/21681 (PCT/US94/03019); GDF-9: WO 94/15966 (PCT/US94/00685); GDF-10: WO 95/10539 (PCT/US94/11440); GDF-11: WO 96/01845 (PCT/US95/08543); BMP-15: WO 96/36710 (PCT/US96/06540); MP121: WO 96/01316 (PCT/EP95/02552); GDF-5 (CDMP-1, MP52): WO 94/15949 (PCT/US94/00657) and WO 96/14335 (PCT/US94/12814) and WO 93/16099 (PCT/EP93/00350); GDF-6 (CDMP-2, BMP-13): WO 95/01801 (PCT/US94/07762) and WO 96/14335 and WO 95/10635 (PCT/US94/14030); GDF-7 (CDMP-3, BMP-12): WO 95/10802 (PCT/US94/07799) and WO 95/10635 (PCT/US94/14030). In another embodiment, useful proteins include biologically active biosynthetic constructs, including novel biosynthetic morphogenic proteins and chimeric proteins designed using sequences from two or more known morphogens. See also the biosynthetic constructs disclosed in U.S. Pat. 5,011,691 (*e.g.*, COP-1, COP-3, COP-4, COP-5, COP-7, and COP-16). The disclosure of all cited references describing morphogens and other related proteins are incorporated herein by reference.

In certain preferred embodiments, useful morphogenic proteins include those in which the amino acid sequences comprise a sequence sharing at least 70% amino

acid sequence homology (identity or conserved substitution), and preferably 80%, 85%, 90%, 95% or 99% homology, with a reference morphogenic protein selected from the exemplary naturally occurring morphogenic proteins listed herein. Preferably, the reference protein is human OP-1, and the reference sequence thereof 5 is the C-terminal seven cysteine skeleton present in osteogenically active forms of human OP-1, residues 330-431 of SEQ ID NO: 3 (or residues 38-139 of SEQ ID NO: 1). Useful morphogenic proteins accordingly include allelic, phylogenetic counterpart and other variants of the preferred reference sequence, whether naturally occurring or biosynthetically produced (*e.g.*, including "muteins" or "mutant 10 proteins"), as well as novel members of the general morphogenic family of proteins including those set forth and identified above. Certain particularly preferred morphogenic polypeptides share at least 50% amino acid identity with the preferred reference sequence of human OP-1, or any of the other morphogens described above, still more preferably at least 55%, 60%, 65%, 70%, 80%, 85%, 90%, 95%, 15 99% or more amino acid identity therewith.

FIG. 28 recites the percent amino acid sequence homology and percent identity within the C-terminal seven cysteine skeletons of various representative members of the TGF- $\beta$  superfamily, using OP-1 as the reference sequence. The percent homologies recited in the figure are determined by aligning the sequences 20 using the MegaAlign Program (DNAstar, Inc.). Insertions and deletions from the reference morphogen sequence (the C-terminal, biologically active seven-cysteine skeleton of hOP-1) are ignored for purposes of calculation (details see below).

As is apparent to one of ordinary skill in the art reviewing the sequences for the proteins listed in FIG. 28, significant amino acid changes can be made from the 25 reference sequence while retaining substantial morphogenic activity. Moreover, GDF-1 contains a four amino acid insert (Gly-Gly-Pro-Pro, SEQ ID NO: 31) between the two residues corresponding to residue 372 and 373 of OP-1 (SEQ ID NO: 3). Similarly, BMP3 has a "extra" residue, a valine, inserted between the two residues corresponding to residues 385 and 386 of hOP-1 (SEQ ID NO: 3). Also, 30 BMP2 and BMP4 are both "missing" the amino acid residue corresponding to

residue 389 of OP-1 (SEQ ID NO: 3). None of these "deviations" from the reference sequence appear to interfere substantially with biological activity.

In other preferred embodiments, the family of morphogenic polypeptides useful in the present invention, and members thereof, are defined by a generic amino acid sequence. For example, Generic Sequence 1 (SEQ ID NO: 24) and Generic Sequence 2 (SEQ ID NO: 25) disclosed below, encompass the observed variations between preferred protein family members identified to date, including at least OP-1, OP-2, OP-3, CBMP2A, CBMP2B, BMP3, 60A, DPP, Vgl, BMP5, BMP6; Vgr-1, and GDF-1. The amino acid sequences for these proteins are described herein and/or in the art, as summarized above. The generic sequences include both the amino acid identity shared by these sequences in the C-terminal skeleton, defined by the six and seven cysteine skeletons (Generic Sequences 1 and 2, respectively), as well as alternative residues for the variable positions within the sequence. The generic sequences provide an appropriate cysteine skeleton where inter- or intra-molecular disulfide bonds can form, and contain certain critical amino acids likely to influence the tertiary structure of the folded proteins. In addition, the generic sequences allow for an additional cysteine at position 36 (Generic Sequence 1) or position 41 (Generic Sequence 2), thereby encompassing the morphogenically active sequences of OP-2 and OP-3.

20 Generic Sequence 1 (SEQ ID NO: 24)

			Leu	Xaa	Xaa	Xaa	Phe	Xaa	Xaa
			1				5		
	Xaa	Gly	Trp	Xaa	Xaa	Xaa	Xaa	Xaa	Pro
25			10				15		
	Xaa	Xaa	Xaa	Xaa	Ala	Xaa	Tyr	Cys	Gly
			20				25		
	Xaa	Cys	Xaa	Xaa	Pro	Xaa	Xaa	Xaa	Xaa
			30				35		

Xaa	Xaa	Xaa	Asn	His	Ala	Xaa	Xaa	Xaa	Xaa	
			40				45			
Xaa										
			50				55			
5	Xaa	Xaa	Xaa	Cys	Cys	Xaa	Pro	Xaa	Xaa	Xaa
			60				65			
Xaa	Xaa	Xaa	Xaa	Xaa	Leu	Xaa	Xaa	Xaa	Xaa	
			70				75			
Xaa	Xaa	Xaa	Val	Xaa	Leu	Xaa	Xaa	Xaa	Xaa	
10			80				85			
Xaa	Met	Xaa	Val	Xaa	Xaa	Cys	Xaa	Cys	Xaa	
			90				95			

wherein each Xaa independently is selected from a group of one or more specified amino acids defined as follows: "Res." means "residue" and Xaa at res. 2 = (Tyr or Lys); Xaa at res. 3 = Val or Ile); Xaa at res. 4 = (Ser, Asp or Glu); Xaa at res. 6 = (Arg, Gln, Ser, Lys or Ala); Xaa at res. 7 = (Asp or Glu); Xaa at res. 8 = (Leu, Val or Ile); Xaa at res. 11 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res. 12 = (Asp, Arg, Asn or Glu); Xaa at res. 13 = (Trp or Ser); Xaa at res. 14 = (Ile or Val); Xaa at res. 15 = (Ile or Val); Xaa at res. 16 (Ala or Ser); Xaa at res. 18 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res. 19 = (Gly or Ser); Xaa at res. 20 = (Tyr or Phe); Xaa at res. 21 = (Ala, Ser, Asp, Met, His, Gln, Leu or Gly); Xaa at res. 23 = (Tyr, Asn or Phe); Xaa at res. 26 = (Glu, His, Tyr, Asp, Gln, Ala or Ser); Xaa at res. 28 = (Glu, Lys, Asp, Gln or Ala); Xaa at res. 30 = (Ala, Ser, Pro, Gln, Ile or Asn); Xaa at res. 31 = (Phe, Leu or Tyr); Xaa at res. 33 = (Leu, Val or Met); Xaa at res.

34 = (Asn, Asp, Ala, Thr or Pro); Xaa at res. 35 = (Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res. 36 = (Tyr, Cys, His, Ser or Ile); Xaa at res. 37 = (Met; Phe, Gly or Leu); Xaa at res. 38 = (Asn, Ser or Lys); Xaa at res. 39 = (Ala, Ser, Gly or Pro); Xaa at res. 40 = (Thr, Leu or Ser); Xaa at res. 44 = (Ile, Val or Thr); Xaa at res. 45  
5 = (Val, Leu, Met or Ile); Xaa at res. 46 = (Gln or Arg); Xaa at res. 47 = (Thr, Ala or Ser); Xaa at res. 48 = (Leu or Ile); Xaa at res. 49 = (Val or Met); Xaa at res. 50 = (His, Asn or Arg); Xaa at res. 51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.  
52 = (Ile, Met, Asn, Ala, Val, Gly or Leu); Xaa at res. 53 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at res. 54 = (Pro, Ser or Val); Xaa at res. 55 = (Glu, Asp, Asn,  
10 Gly, Val, Pro or Lys); Xaa at res. 56 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Gly, Ile or His); Xaa at res. 57 = (Val, Ala or Ile); Xaa at res. 58 = (Pro or Asp); Xaa at res.  
59 = (Lys, Leu or Glu); Xaa at res. 60 = (Pro, Val or Ala); Xaa at res. 63 = (Ala or Val); Xaa at res. 65 = (Thr, Ala or Glu); Xaa at res. 66 = (Gln, Lys, Arg or Glu);  
Xaa at res. 67 = (Leu, Met or Val); Xaa at res. 68 = (Asn, Ser, Asp or Gly); Xaa at  
15 res. 69 = (Ala, Pro or Ser); Xaa at res. 70 = (Ile, Thr, Val or Leu); Xaa at res. 71 = (Ser, Ala or Pro); Xaa at res. 72 = (Val, Leu, Met or Ile); Xaa at res. 74 = (Tyr or Phe); Xaa at res. 75 = (Phe, Tyr, Leu or His); Xaa at res. 76 = (Asp, Asn or Leu);  
Xaa at res. 77 = (Asp, Glu, Asn, Arg or Ser); Xaa at res. 78 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res. 79 = (Ser, Asn, Asp, Glu or Lys); Xaa at res. 80 = (Asn, Thr or  
20 Lys); Xaa at res. 82 = (Ile, Val or Asn); Xaa at res. 84 = (Lys or Arg); Xaa at res.  
85 = (Lys, Asn, Gln, His, Arg or Val); Xaa at res. 86 = (Tyr, Glu or His); Xaa at  
res. 87 = (Arg, Gln, Glu or Pro); Xaa at res. 88 = (Asn, Glu, Trp or Asp); Xaa at  
res. 90 = (Val, Thr, Ala or Ile); Xaa at res. 92 = (Arg, Lys, Val, Asp, Gln or Glu);

Xaa at res. 93 = (Ala, Gly, Glu or Ser); Xaa at res. 95 = (Gly or Ala) and Xaa at res. 97 = (His or Arg).

Generic Sequence 2 (SEQ ID NO: 25) includes all of Generic Sequence 1 (SEQ ID NO: 24) and in addition includes the following sequence (SEQ ID NO: 26)  
5 at its N-terminus:

**SEQ ID NO: 26**

Cys	Xaa	Xaa	Xaa	Xaa
1				5

10 Accordingly, beginning with residue 7, each "Xaa" in Generic Sequence 2 is a specified amino acid defined as for Generic Sequence 1, with the distinction that each residue number described for Generic Sequence 1 is shifted by five in Generic Sequence 2. Thus, "Xaa. at res. 2 = (Tyr or Lys)" in Generic Sequence 1 refers to Xaa at res. 7 in Generic Sequence 2. In Generic Sequence 2, Xaa at res. 2 = (Lys, Arg, Ala or Gln); Xaa at res. 3 = (Lys, Arg or Met); Xaa at res. 4 = (His, Arg or Gln); and Xaa at res. 5 = (Glu, Ser, His, Gly, Arg, Pro, Thr, or Tyr).  
15

In another embodiment, useful osteogenic proteins include those defined by Generic Sequences 3 and 4 (SEQ ID NOs: 27 and 28, respectively), described herein above. Specifically, Generic Sequences 3 and 4 are composite amino acid,  
20 sequences of the following proteins: human OP-1, human OP-2, human OP-3, human BMP2, human BMP3, human BMP4, human BMP5, human BMP6, human BMP8, human BMP9, human BMP10, human BMP11, Drosophila 60A, Xenopus Vg1, sea urchin UNIVIN, human CBMP1 (mouse GDF-5), human CBMP2 (mouse GDF-6, human BMP13), human CBMP3 (mouse GDF-7, human BMP12), mouse GDF3, human GDF-1, mouse GDF-1, chicken DORSALIN, Drosophila DPP, Drosophila SCREW, mouse NODAL, mouse GDF-8, human GDF-8, mouse GDF-9, mouse GDF-10, human GDF-11, mouse GDF-11, human BMP15, and rat BMP3b.  
25 Like Generic Sequence 1, Generic Sequence 3 accommodates the C-terminal six cysteine skeleton and, like Generic Sequence 2, Generic Sequence 4 accommodates  
30 the seven cysteine skeleton.

**Generic Sequence 3 (SEQ ID NO: 27)**

Xaa									
1				5					10
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Pro	Xaa	Xaa	Xaa
				15					20
Xaa	Xaa	Xaa	Xaa	Cys	Xaa	Gly	Xaa	Cys	Xaa
				25					30
Xaa									
				35					40
Xaa									
				45					50
Xaa									
				55					60
Xaa	Cys	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
				65					70
Xaa	Xaa	Leu	Xaa						
				75					80
Xaa									
				85					90
Xaa	Xaa	Xaa	Cys	Xaa	Cys	Xaa			
				95					

wherein each Xaa is independently selected from a group of one or more specified amino acids defined as follows: "Res." means "residue" and Xaa at res. 1 = (Phe,

- 5 Leu or Glu); Xaa at res. 2 = (Tyr, Phe, His, Arg, Thr, Lys, Gln, Val or Glu); Xaa at res. 3 = (Val, Ile, Leu or Asp); Xaa at res. 4 = (Ser, Asp, Glu, Asn or Phe); Xaa at res. 5 = (Phe or Glu); Xaa at res. 6 = (Arg, Gln, Lys, Ser, Glu, Ala or Asn); Xaa at res. 7 = (Asp, Glu, Leu, Ala or Gln); Xaa at res. 8 = (Leu, Val, Met, Ile or Phe); Xaa at res. 9 = (Gly, His or Lys); Xaa at res. 10 = (Trp or Met); Xaa at res. 11 =
- 10 (Gln, Leu, His, Glu, Asn, Asp, Ser or Gly); Xaa at res. 12 = (Asp, Asn, Ser, Lys, Arg, Glu or His); Xaa at res. 13 = (Trp or Ser); Xaa at res. 14 = (Ile or Val); Xaa at res. 15 = (Ile or Val); Xaa at res. 16 = (Ala, Ser, Tyr or Trp); Xaa at res. 18 = (Glu, Lys, Gln, Met, Pro, Leu, Arg, His or Lys); Xaa at res. 19 = (Gly, Glu, Asp, Lys, Ser, Gln, Arg or Phe); Xaa at res. 20 = (Tyr or Phe); Xaa at res. 21 = (Ala, Ser,

Gly, Met, Gln, His, Glu, Asp, Leu, Asn, Lys or Thr); Xaa at res. 22 = (Ala or Pro);  
Xaa at res. 23 = (Tyr, Phe, Asn, Ala or Arg); Xaa at res. 24 = (Tyr, His, Glu, Phe  
or Arg); Xaa at res. 26 = (Glu, Asp, Ala, Ser, Tyr, His, Lys, Arg, Gln or Gly); Xaa  
at res. 28 = (Glu, Asp, Leu, Val, Lys, Gly, Thr, Ala or Gln); Xaa at res. 30 = (Ala,  
5 Ser, Ile, Asn, Pro, Glu, Asp, Phe, Gln or Leu); Xaa at res. 31 = (Phe, Tyr, Leu,  
Asn, Gly or Arg); Xaa at res. 32 = (Pro, Ser, Ala or Val); Xaa at res. 33 = (Leu,  
Met, Glu, Phe or Val); Xaa at res. 34 = (Asn, Asp, Thr, Gly, Ala, Arg, Leu or Pro);  
Xaa at res. 35 = (Ser, Ala, Glu, Asp, Thr, Leu, Lys, Gln or His); Xaa at res. 36 =  
(Tyr, His, Cys, Ile, Arg, Asp, Asn, Lys, Ser, Glu or Gly); Xaa at res. 37 = (Met,  
10 Leu, Phe, Val, Gly or Tyr); Xaa at res. 38 = (Asn, Glu, Thr, Pro, Lys, His, Gly,  
Met, Val or Arg); Xaa at res. 39 = (Ala, Ser, Gly, Pro or Phe); Xaa at res. 40 =  
(Thr, Ser, Leu, Pro, His or Met); Xaa at res. 41 = (Asn, Lys, Val, Thr or Gln); Xaa  
at res. 42 = (His, Tyr or Lys); Xaa at res. 43 = (Ala, Thr, Leu or Tyr); Xaa at res.  
44 = (Ile, Thr, Val, Phe, Tyr, Met or Pro); Xaa at res. 45 = (Val, Leu, Met, Ile or  
15 His); Xaa at res. 46 = (Gln, Arg or Thr); Xaa at res. 47 = (Thr, Ser, Ala, Asn or  
His); Xaa at res. 48 = (Leu, Asn or Ile); Xaa at res. 49 = (Val, Met, Leu, Pro or  
Ile); Xaa at res. 50 = (His, Asn, Arg, Lys, Tyr or Gln); Xaa at res. 51 = (Phe, Leu,  
Ser, Asn, Met, Ala, Arg, Glu, Gly or Gln); Xaa at res. 52 = (Ile, Met, Leu, Val,  
Lys, Gln, Ala or Tyr); Xaa at res. 53 = (Asn, Phe, Lys, Glu, Asp, Ala, Gln, Gly,  
20 Leu or Val); Xaa at res. 54 = (Pro, Asn, Ser, Val or Asp); Xaa at res. 55 = (Glu,  
Asp, Asn, Lys, Arg, Ser, Gly, Thr, Gln, Pro or His); Xaa at res. 56 = (Thr, His,  
Tyr, Ala, Ile, Lys, Asp, Ser, Gly or Arg); Xaa at res. 57 = (Val, Ile, Thr, Ala, Leu  
or Ser); Xaa at res. 58 = (Pro, Gly, Ser, Asp or Ala); Xaa at res. 59 = (Lys, Leu,  
Pro, Ala, Ser, Glu, Arg or Gly); Xaa at res.: 60 = (Pro, Ala, Val, Thr or Ser); Xaa

at res. 61 = (Cys, Val or Ser); Xaa at res. 63 = (Ala, Val or Thr); Xaa at res. 65 = (Thr, Ala, Glu, Val, Gly, Asp or Tyr); Xaa at res. 66 = (Gln, Lys, Glu, Arg or Val); Xaa at res. 67 = (Leu, Met, Thr or Tyr); Xaa at res. 68 = (Asn, Ser, Gly, Thr, Asp, Glu, Lys or Val); Xaa at res. 69 = (Ala, Pro, Gly or Ser); Xaa at res. 70 = (Ile, Thr, 5 Leu or Val); Xaa at res. 71 = (Ser, Pro, Ala, Thr, Asn or Gly); Xaa at res. 2 = (Val, Ile, Leu or Met); Xaa at res. 74 = (Tyr, Phe, Arg, Thr, Tyr or Met); Xaa at res. 75 = (Phe, Tyr, His, Leu, Ile, Lys, Gln or Val); Xaa at res. 76 = (Asp, Leu, Asn or Glu); Xaa at res. 77 = (Asp, Ser, Arg, Asn, Glu, Ala, Lys, Gly or Pro); Xaa at res. 78 = (Ser, Asn, Asp, Tyr, Ala, Gly, Gln, Met, Glu, Asn or Lys); Xaa at res. 79 = (Ser, 10 Asn, Glu, Asp, Val, Lys, Gly, Gln or Arg); Xaa at res. 80 = (Asn, Lys, Thr, Pro, Val, Ile, Arg; Ser or Gln); Xaa at res. 81 = (Val, Ile, Thr or Ala); Xaa at res. 82 = (Ile, Asn, Val, Leu, Tyr, Asp or Ala); Xaa at res. 83 = (Leu, Tyr, Lys or Ile); Xaa at res. 84 = (Lys, Arg, Asn, Tyr, Phe, Thr, Glu or Gly); Xaa at res. 85 = (Lys, Arg, His, Gln, Asn, Glu or Val); Xaa at res. 86 = (Tyr, His, Glu or Ile); Xaa at res. 87 = (Arg, Glu, Gln, Pro or Lys); Xaa at res. 88 = (Asn, Asp, Ala, Glu, Gly or Lys); 15 Xaa at res. 89 = (Met or Ala); Xaa at res. 90 = (Val, Ile, Ala, Thr, Ser or Lys); Xaa at res. 91 = (Val or Ala); Xaa at res. 92 = (Arg, Lys, Gln, Asp, Glu, Val, Ala, Ser or Thr); Xaa at res. 93 = (Ala, Ser, Glu, Gly, Arg or Thr); Xaa at res. 95 = (Gly, Ala or Thr); Xaa at res. 97 = (His, Arg, Gly, Leu or Ser). Further, after res. 53 in 20 rBMP-3b and mGDF-10 there is an Ile; after res. 54 in GDF-1 there is a T; after res. 54 in BMP3 there is a V; after res. 78 in BMP8 and Dorsalin there is a G; after res. 37 in hGDF-1 there is Pro, Gly, Gly, Pro.

Generic Sequence 4 (SEQ ID NO: 28) includes all of Generic Sequence 3 (SEQ ID NO: 27) and in addition includes the following sequence (SEQ ID NO: 26) 25 at its N-terminus:

**SEQ ID NO: 26**

Cys Xaa Xaa Xaa Xaa

1

5

Accordingly, beginning with residue 6, each "Xaa" in Generic Sequence 4 is  
5 a specified amino acid defined as for Generic Sequence 3, with the distinction that  
each residue number described for Generic Sequence 3 is shifted by five in Generic  
Sequence 4. Thus, "Xaa at res. 1 = (Tyr, Phe, His, Arg, Thr, Lys, Gln, Val or Glu)"  
in Generic Sequence 3 refers to Xaa at res. 6 in Generic Sequence 4. In Generic  
Sequence 4, Xaa at res. 2 = (Lys, Arg, Gln, Ser, His, Glu, Ala, or Cys); Xaa at res. 3  
10 = (Lys, Arg, Met, Lys, Thr, Leu, Tyr, or Ala); Xaa at res. 4 = (His, Gln, Arg, Lys,  
Thr, Leu, Val, Pro, or Tyr); and Xaa at res. 5 = (Gln, Thr, His, Arg, Pro, Ser, Ala,  
Gln, Asn, Tyr, Lys, Asp, or Leu).

Based upon alignment of the naturally occurring morphogens within the  
definition of Generic Sequence 4, it should be clear that gaps and/or insertions of  
15 one or more amino acid residues can be tolerated (without abrogating or  
substantially impairing biological activity) at least between or involving residues 11-  
12, 42-43, 59-60, 68-69 and 83-84.

Particularly useful sequences for use as morphogens in this invention include  
the C-terminal domains, e.g., the C-terminal 96-102 amino acid residues of Vgl,  
20 Vgr-1, DPP, OP-1, OP-2, CBMP2A, CBMP2B, GDF-1 (see Table II, below, and  
SEQ ID NOS: 1-13), as well as proteins comprising the C-terminal domains of 60A,  
BMP3, BMP5 and BMP6 (see SEQ ID NOS: 12, 14-16), all of which include at least  
the conserved six or seven cysteine skeleton. In addition, biosynthetic constructs  
designed from the generic sequences, such as COP-1, 3-5, 7, 16, disclosed in U.S.  
25 Pat. No. 5,011,691, also are useful. Other sequences include the inhibins/activin  
proteins (see, for example, U.S. Pat. Nos. 4,968,590 and 5,011,691). Accordingly,  
other useful sequences are those sharing at least 70% amino acid sequence  
homology or 50% identity, and preferably 80% homology or 70% identity with any  
of the sequences above. These are anticipated to include allelic and species variants  
30 and mutants, and biosynthetic muteins, as well as novel members of this

morphogenic family of proteins. Particularly envisioned in the family of related proteins are those proteins exhibiting morphogenic activity and wherein the amino acid changes from the preferred sequences include conservative changes.

Information regarding conserved amino acid changes are well-known in the art. For example, Dayhoff et al. described in *Atlas of Protein Sequence and Structure*; vol. 5, Suppl. 3, pp. 345-362, (M.O. Dayhoff, ed., Nat'l BioMed. Research Fdn., Washington, D.C. 1978) that certain amino acids substitutions among evolutionary conserved proteins occur at higher than expected frequency than random chance would allow. Thus, conserved amino acid substitutions can be determined according to Figure 84 (*supra*). As used herein, potentially useful sequences are aligned with a known morphogen sequence using the method of Needleman et al. ((1970) *J. Mol. Biol.* 48:443-453) and identities calculated by the MegaAlign program (DNAsstar, Inc.).

Table II, set forth below, compares the amino acid sequences of the active regions of native proteins that have been identified as morphogens, including human OP-1 (hOP-1, SEQ ID NOs: 1-3), mouse OP-1 (mOP-1, SEQ ID NOs: 4 and 19), human and mouse OP-2 (SEQ ID NOs: 5, 6, 21, and 23), CBMP2A (SEQ ID NO: 10), CBMP2B (SEQ ID NO: 11), BMP3 (SEQ ID NO: 12), DPP (from *Drosophila*, SEQ ID NO: 7), Vgl (from *Xenopus*, SEQ ID NO: 8), Vgr-1 (from mouse, SEQ ID NO: 9), GDF-1 (from mouse, SEQ ID NOs: 13), 60A protein (from *Drosophila*, SEQ ID NOs: 14), BMP5 (SEQ ID NO: 15) and BMP6 (SEQ ID NO: 16). The sequences are aligned essentially following the method of Needleman et al. (1970) *J. Mol. Biol.*, 48: 443-453, calculated using the Align Program (DNAsstar, Inc.) In the table, three dots indicates that the amino acid in that position is the same as the amino acid in hOP-1. Three dashes indicates that no amino acid is present in that position, and are included for purposes of illustrating homologies. For example, amino acid residue 60 of CBMP-2A and CBMP-2B is "missing". Of course, both these amino acid sequences in this region comprise Asn-Ser (residues 58, 59), with CBMP-2A then comprising Lys and Ile, whereas CBMP-2B comprises Ser and Ile.

30

**TABLE II**

hOP-1	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val
mOP-1	...	...	...	...	...	...	...	...
hOP-2	...	Arg	Arg	...	...	...	...	...
mOP-2	...	Arg	Arg	...	...	...	...	...
DPP	...	Arg	Arg	...	Ser	...	...	...
Vgl	...	...	Lys	Arg	His	...	...	...
Vgr-1	...	...	...	...	Gly	...	...	...
CBMP-2A	...	...	Arg	...	Pro	...	...	...
CBMP-2B	...	Arg	Arg	...	Ser	...	...	...
BMP3	...	Ala	Arg	Arg	Tyr	...	Lys	...
GDF-1	...	Arg	Ala	Arg	Arg	...	...	...
60A	...	Gln	Met	Glu	Thr	...	...	...
BMP5	...	...	...	...	...	...	...	...
BMP6	...	Arg	...	...	...	...	...	...
	1				5			

hOP-1	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	...	Gln	...	...	...	...	Leu	...
mOP-2	Ser	...	...	...	...	...	...	Leu	...
DPP	Asp	...	Ser	...	Val	...	...	Asp	...
Vgl	Glu	...	Lys	...	Val	...	...	...	Asn
Vgr-1	...	...	Gln	...	Val	...	...	...	...
CBMP-2A	Asp	...	Ser	...	Val	...	...	Asn	...
CBMP-2B	Asp	...	Ser	...	Val	...	...	Asn	...
BMP3	Asp	...	Ala	...	Ile	...	...	Ser	Glu
GDF-1	...	...	...	Glu	Val	...	...	His	Arg
60A	Asp	...	Lys	...	...	...	...	His	...
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	Gln	...	...	...	...	...	...
	10						15		

hOP-1	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	Val	...	...	...	Gln	...	...	Ser
mOP-2	...	Val	...	...	...	Gln	...	...	Ser
DPP	...	...	Val	...	...	Leu	...	...	Asp
Vgl	...	Val	...	...	...	Gln	...	...	Met
Vgr-1	...	...	...	...	...	Lys	...	...	...
CBMP-2A	...	...	Val	...	...	Pro	...	...	His
CBMP-2B	...	...	Val	...	...	Pro	...	...	Gln
BMP3	...	...	...	Ser	...	Lys	Ser	Phe	Asp
GDF-1	...	Val	...	...	...	Arg	...	Phe	Leu
60A	...	...	...	...	...	...	...	...	Gly
BMP5	...	...	...	...	...	...	...	...	...

BMP6	...	...	...	...	...	Lys	...	...	...
			20				25		
hOP-1	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	...	...	...	...	...	...	...	Ser
mOP-2	...	...	...	...	...	...	...	...	...
DPP	...	...	...	...	His	...	Lys	...	Pro
Vgl	...	Asn	...	...	Tyr	...	...	...	Pro
Vgr-1	...	Asn	...	...	Asp	...	...	...	Ser
CBMP-2A	...	Phe	...	...	His	...	Glu	...	Pro
CBMP-2B	...	Phe	...	...	His	...	Asp	...	Pro
BMP3	...	...	...	...	Ser	...	Ala	...	Gln
GDF-1	...	Asn	...	...	Gln	...	Gln	...	...
60A	...	Phe	...	...	Ser	...	...	...	Asn
BMP5	...	Phe	...	...	Asp	...	...	...	Ser
BMP6	...	Asn	...	...	Asp	...	...	...	Ser
			30		.				35
hOP-1	Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	...	...	Asp	...	Cys	...	...	...
mOP-2	...	...	...	Asp	...	Cys	...	...	...
DPP	...	...	...	Ala	Asp	His	Phe	...	Ser
Vgl	Tyr	...	...	Thr	Glu	Ile	Leu	...	Gly
Vgr-1	...	...	...	...	Ala	His	...	...	...
CBMP-2A	...	...	...	Ala	Asp	His	Leu	...	Ser
CBMP-2B	...	...	...	Ala	Asp	His	Leu	...	Ser
BMP3	Leu	...	Val	Ala	Leu	Ser	Gly	Ser†	...
GDF-1	...	...	Met	Pro	Lys	Ser	Leu	Lys	Pro
60A	...	...	...	...	Ala	His	...	...	...
BMP5	...	...	...	...	Ala	His	Met	...	...
BMP6	...	...	...	...	Ala	His	Met	...	...
			40						
hOP-1	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	...	...	...	...	Leu	...	Ser	...
mOP-2	...	...	...	...	...	Leu	...	Ser	...
DPP	...	...	...	...	...	Val	...	...	...
Vgl	Ser	...	...	...	...	Leu	...	...	...
Vgr-1	...	...	...	...	...	...	...	...	...
CBMP-2A	...	...	...	...	...	...	...	...	...
CBMP-2B	...	...	...	...	...	...	...	...	...
BMP3	Ser	...	...	...	Thr	Ile	...	Ser	Ile
GDF-1	Leu	...	...	...	Val	Leu	Arg	Ala	...

60A	...	...	...	...	...	...	...	...	...	...
BMP5	...	...	...	...	...	...	...	...	...	...
BMP6	...	...	...	...	...	...	...	...	...	...
	45						50			
hOP-1	Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val	
mOP-1	...	...	...	...	...	...	Asp	...	...	
hOP-2	...	His	Leu	Met	Lys	...	Asn	Ala	...	
mOP-2	...	His	Leu	Met	Lys	...	Asp	Val	...	
DPP	...	Asn	Asn	Asn	...	...	Gly	Lys	...	
Vgl	...	...	Ser	...	Glu	...	...	Asp	Ile	
Vgr-1	...	...	Val	Met	...	...	...	Tyr	...	
CBMP-2A	...	Asn	Ser	Val	...	Ser	---	Lys	Ile	
CBMP-2B	...	Asn	Ser	Val	...	Ser	---	Ser	Ile	
BMP3	...	Arg	Ala†	Gly	Val	Val	Pro	Gly	Ile	
GDF-1	Met	...	Ala	Ala	Ala	...	Gly	Ala	Ala	
60A	...	...	Leu	Leu	Glu	...	Lys	Lys	...	
BMP5	...	...	Leu	Met	Phe	...	Asp	His	...	
BMP6	...	...	Leu	Met	...	...	...	Tyr	...	
	55						60			
hOP-1	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln	
mOP-1	...	...	...	...	...	...	...	...	...	
hOP-2	...	...	Ala	...	...	...	...	...	Lys	
mOP-2	...	...	Ala	...	...	...	...	...	Lys	
DPP	...	...	Ala	...	...	Val	...	...	...	
Vgl	...	Leu	...	...	...	Val	...	...	Lys	
Vgr-1	...	...	...	...	...	...	...	...	Lys	
CBMP-2A	...	...	Ala	...	...	Val	...	...	Glu	
CBMP-2B	...	...	Ala	...	...	Val	...	...	Glu	
BMP3	...	Glu	...	...	...	Val	...	Glu	Lys	
GDF-1	Asp	Leu	...	...	...	Val	...	Ala	Arg	
60A	...	...	...	...	...	...	...	...	Arg	
BMP5	...	...	...	...	...	...	...	...	Lys	
BMP6	...	...	...	...	...	...	...	...	Lys	
	65						70			
hOP-1	Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe	
mOP-1	...	...	...	...	...	...	...	...	...	
hOP-2	...	Ser	...	Thr	...	...	...	...	Tyr	
mOP-2	...	Ser	...	Thr	...	...	...	...	Tyr	
Vgl	Met	Ser	Pro	...	...	Met	...	Phe	Tyr	
Vgr-1	Val	...	...	...	...	...	...	...	...	
DPP	...	Asn	Ser	Val	Ala	Met	...	...	Leu	
CBMP-2A	...	Ser	...	...	...	Met	...	...	Leu	
CBMP-2B	...	Ser	...	...	...	Met	...	...	Leu	

BMP3	Met	Ser	Ser	Leu	...	Ile	...	Phe	Tyr
GDF-1	...	Ser	Pro	...	...	...	...	Phe	...
60A	...	Gly	...	Leu	Pro	...	...	...	His
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	...	...	...	...	...	...	...
					75				80
hOP-1	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	Ser	...	Asn	...	...	...	...	Arg
mOP-2	...	Ser	...	Asn	...	...	...	...	Arg
DPP	Asn	...	Gln	...	Thr	...	Val	...	...
Vgl	...	Asn	Asn	Asp	...	...	Val	...	Arg
Vgr-1	...	...	Asn	...	...	...	...	...	...
CBMP-2A	...	Glu	Asn	Glu	Lys	...	Val	...	...
CBMP-2B	...	Glu	Tyr	Asp	Lys	...	Val	...	...
BMP3	...	Glu	Asn	Lys	...	...	Val	...	...
GDF-1	...	Asn	...	Asp	...	...	Val	...	Arg
60A	Leu	Asn	Asp	Glu	...	...	Asn	...	...
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	Asn	...	...	...	...	...	...
					85				
hOP-1	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg	
mOP-1	...	...	...	...	...	...	...	...	
hOP-2	...	His	...	...	...	...	...	Lys	
mOP-2	...	His	...	...	...	...	...	Lys	
DPP	Asn	...	Gln	Glu	...	Thr	...	Val	
Vgl	His	...	Glu	...	...	Ala	...	Asp	
Vgr-1	...	...	...	...	...	...	...	...	
CBMP-2A	Asn	...	Gln	Asp	...	...	...	Glu	
CBMP-2B	Asn	...	Gln	Glu	...	...	...	Glu	
BMP3	Val	...	Pro	...	...	Thr	...	Glu	
GDF-1	Gln	...	Glu	Asp	...	...	...	Asp	
60A	...	...	...	...	...	Ile	...	Lys	
BMP5	...	...	...	...	...	...	...	...	
BMP6	...	...	...	Trp	...	...	...	...	
		90					95		
hOP-1	Ala	Cys	Gly	Cys	His				
mOP-1	...	...	...	...	...				
hOP-2	...	...	...	...	...				
mOP-2	...	...	...	...	...				
DPP	Gly	...	...	...	Arg				
Vgl	Glu	...	...	...	Arg				
Vgr-1	...	...	...	...	...				

CBMP-2A	Gly	...	...	...	Arg
CBMP-2B	Gly	...	...	...	Arg
BMP3	Ser	...	Ala	...	Arg
GDF-1	Glu	...	...	...	Arg
60A	Ser	...	...	...	...
BMP5	Ser	...	...	...	...
BMP6	...	...	...	...	...
				100	

† Between residues 56 and 57 of BMP3 is a Val residue; between residues 43 and 44 of GDF-1 lies the amino acid sequence Gly-Gly-Pro-Pro.

As is apparent from the foregoing amino acid sequence comparisons,  
5 significant amino acid changes can be made within the generic sequences while  
retaining the morphogenic activity.

As noted above, certain preferred morphogenic polypeptide sequences useful  
in this invention have greater than 50% identity, preferably greater than 55%, 60%,  
65%, 70%, 80%, 85%, 90%, 95% or even 99% identity, with the amino acid  
10 sequence defining the preferred reference sequence of hOP-1 (especially the  
conserved six-seven cysteine skeleton of hOP-1, e.g., residues 39-139 of SEQ ID  
No: 1), or equivalent regions from other morphogens described in the application.  
These particularly preferred sequences include allelic and phylogenetic counterpart  
variants of the OP-1 and OP-2 proteins, including the *Drosophila* 60A protein, as  
15 well as the closely related proteins BMP5, BMP6 and Vgr-1. Accordingly, in certain  
particularly preferred embodiments, useful morphogenic proteins include active  
proteins comprising pairs of polypeptide chains within the generic amino acid  
sequence herein referred to as "OPX" (SEQ ID NO: 29), which defines the seven  
cysteine skeleton and accommodates the homologies between several identified  
20 variants of OP-1 and OP-2. Accordingly, each "Xaa" at a given position in OPX  
independently is selected from the residues occurring at the corresponding position  
in the C-terminal sequence of mouse or human OP-1 or-OP-2. Specifically, each  
"Xaa" is independently selected from a group of one or more specified amino acids  
as defined below:

Cys 1	Xaa	Xaa	His	Glu 5	Leu	Tyr	Val	Ser	Phe 10	Xaa	Asp	Leu	Gly	Trp 15
Xaa	Asp	Trp	Xaa	Ile 20	Ala	Pro	Xaa	Gly	Tyr 25	Xaa	Ala	Tyr	Tyr	Cys 30
Glu	Gly	Glu	Cys	Xaa 35	Phe	Pro	Leu	Xaa	Ser 40	Xaa	Met	Asn	Ala	Thr 45
Asn	His	Ala	Ile	Xaa 50	Gln	Xaa	Leu	Val	His 55	Xaa	Xaa	Xaa	Pro	Xaa 60
Xaa	Val	Pro	Lys	Xaa 65	Cys	Cys	Ala	Pro	Thr 70	Xaa	Leu	Xaa	Ala	Xaa 75
Ser	Val	Leu	Tyr	Xaa 80	Asp	Xaa	Ser	Xaa	Asn 85	Val	Ile	Leu	Xaa	Lys 90
Xaa	Arg	Asn	Met	Val 95	Xaa	Ala	Cys	Gly	Cys 100	His				

wherein Xaa at res. 2 = (Lys or Arg); Xaa at res. 3 = (Lys or Arg); Xaa at res. 11 = (Arg or Gln); Xaa at res. 16 = (Gln or Leu); Xaa. at res. 19 = (Ile or Val); Xaa at res. 23 = (Glu or Gln); Xaa at res. 26 = (Ala or Ser); Xaa at res. 35 = (Ala or Ser); Xaa at res. 39 = (Asn or Asp); Xaa at res. 41 = (Tyr or Cys); Xaa at res. 50 = (Val or Leu); Xaa at res. 52 = (Ser or Thr); Xaa at res. 56 = (Phe or Leu); Xaa at res. 57 = (Ile or Met); Xaa at res. 58 = (Asn or Lys); Xaa at res. 60 = (Glu, Asp or Asn); Xaa at res. 61 = (Thr, Ala or Val); Xaa at res. 65 = (Pro or Ala); Xaa at res. 71 = (Gln or Lys); Xaa at res. 73 = (Asn or Ser); Xaa at res. 75 = (Ile or Thr); Xaa at res. 80 = (Phe or Tyr); Xaa at res. 82 = (Asp or Ser); Xaa at res. 84 = (Ser or Asn); 10 Xaa at res. 89 = (Lys or Arg); Xaa at res. 91 = (Tyr or His); and Xaa at res. 97 = (Arg or Lys).

The following patents or publications or patent applications disclose morphogens or formula of useful / active morphogens, the entire contents of which are hereby incorporated by reference herein: EP 601106, and USSN 08/937,755 15 (filed on September 25, 1997).

The morphogens useful in the methods, composition and devices of this invention include proteins comprising any of the polypeptide chains described above, whether isolated from naturally occurring sources, or produced by recombinant DNA or other synthetic techniques, and includes allelic and species

variants of these proteins, naturally occurring or biosynthetic mutants thereof, as well as various truncated and fusion constructs. Deletion or addition mutants also are envisioned to be active, including those which may alter the conserved C-terminal cysteine skeleton, provided that the alteration does not functionally disrupt the 5 relationship of these cysteines in the folded structure. Accordingly, such active forms are considered the equivalent of the specifically described constructs disclosed herein.

The proteins may include forms having varying glycosylation patterns, varying N-termini, a family of related proteins having regions of amino acid 10 sequence homology, and active truncated or mutated forms of native or biosynthetic proteins, produced by expression of recombinant DNA in host cells.

The morphogenic proteins can be expressed from intact or truncated cDNA or from synthetic DNAs in prokaryotic or eukaryotic host cells, and purified, cleaved, refolded, and dimerized to form morphogenically active compositions. 15 Currently preferred host cells include *E. coli* or any suitable mammalian host cells, such as CHO, COS or BSC cells. A detailed description of the morphogens useful in the methods, compositions and devices of this invention is disclosed in copending US patent application Serial Nos. 08/937755, filed September 25, 1997, and issued European Patent EP 601106, the contents of which are all incorporated by reference 20 herein. Thus, in view of this disclosure, skilled genetic engineers can isolate genes from cDNA or genomic libraries of various different species which encode appropriate amino acid sequences, or construct DNAs from oligonucleotides, and then can express them in various types of host cells, including both prokaryotes and eukaryotes, to produce large quantities of active proteins capable of protecting 25 tissues and organs from immune cell-mediated tissue destruction, including substantially inhibiting such damage and/or regenerating the damaged tissue in a variety of mammals, including humans.

In still another preferred embodiment, useful morphogenically active proteins have polypeptide chains with amino acid sequences comprising a sequence 30 encoded by a nucleic acid that hybridizes with DNA or RNA encoding reference morphogen sequences, e.g., C-terminal sequences defining the conserved seven

cysteine skeletons of OP-1, OP-2, BMP2, BMP4, BMP5, BMP6, 60A, GDF-3, GDF-5, GDF-6, GDF-7 and the like. As used herein, high stringency hybridization conditions are defined as hybridization according to known techniques in 40% formamide, 5 X SSPE, 5 X Denhardt's Solution, and 0.1% SDS at 37 °C overnight, 5 and washing in 0.1 X SSPE, 0.1% SDS at 50 °C. Standard stringency conditions are well characterized in standard molecular biology cloning texts. See, for example, MOLECULAR CLONING-A LABORATORY MANUAL, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989); DNA CLONING, Volumes I and II (D.N. Glover ed., 1985); OLIGONUCLEOTIDE SYNTHESIS (M.J. Gait 10 ed., 1984); NUCLEIC ACID HYBRIDIZATION (B. D. Hames & S.J. Higgins eds. 1984); and B. Perbal, A PRACTICAL GUIDE TO MOLECULAR CLONING (1984).

In other embodiments, as an alternative to the administration of a morphogenic protein, an effective amount of an agent competent to stimulate or induce increased endogenous morphogen expression in a mammal may be 15 administered by any of the routes described herein. Such a morphogen inducer may be provided to a mammal, *e.g.*, by systemic administration to the mammal or by direct administration to the neural tissue. A method for identifying and testing inducers (stimulating agents) competent to modulate the levels of endogenous morphogens in a given tissue is described in published applications WO 93/05172 20 and WO 93/05751, each of which is incorporated by reference herein. Briefly, candidate compounds are identified and tested by incubation *in vitro* with test tissue or cells, or a cultured cell line derived therefrom, for a time sufficient to allow the compound to affect the production, *i.e.*, cause the expression and/or secretion, of a morphogen produced by the cells of that tissue. Suitable tissues, or cultured cells of 25 a suitable tissue, are preferably selected from renal epithelium, ovarian tissue, fibroblasts, and osteoblasts.

In yet other embodiments, an agent which acts as an agonist of a morphogen receptor may be administered instead of the morphogen itself. Such an agent may also be referred to as a morphogen "mimic," "mimetic," or "analog." Thus, for 30 example, a small peptide or other molecule which can mimic the activity of a morphogen in binding to and activating the morphogen's receptor- may be employed

as an equivalent of the morphogen. Preferably the agonist is a full agonist, but partial morphogen receptor agonists may also be advantageously employed. Methods of identifying such agonists are known in the art and include assays for compounds which induce morphogen-mediated responses (e.g., induction of differentiation of metanephric mesenchyme, induction of endochondral bone formation). For example, methods of identifying morphogen inducers or agonists of morphogen receptors may be found in U.S.S.N. 08/478,097 filed June 7, 1995; U.S.S.N. 09/791946, filed Feb. 22, 2001; U.S. Pat. No. 5,834,188; U.S. Pat. No. 6,273,598; WO 97/26277; EP 0876401; U.S. Provisional Application No. 5 10 15 10 15 reference.

The OP/BMP family of morphogens of the invention are also characterized by biological activities which may be readily ascertained by those of ordinary skill in the art. Specifically, a morphogen of the present invention is (a) capable of inducing chondrogenesis in the Reddi-Sampath ectopic bone assay (Sampath and Reddi (1981), Proc. Natl. Acad. Sci. USA 78:7599-7603) or a substantially equivalent assay, (b) capable of significantly preventing, inhibiting, delaying or alleviating the progressive loss of renal function in a standard animal model of chronic renal failure, or (c) capable of causing a clinically significant improvement in a standard marker of renal function when administered to a mammal in, or at risk 20 25 of, chronic renal failure.

The Reddi-Sampath ectopic bone assay is well known in the art as an assay of chondrogenic activity. The assay, which can be easily performed, is described and discussed in, for example, Sampath and Reddi (1981), Proc. Natl. Acad. Sci. USA 78: 7599-7603; and function which characterizes chronic renal failure.

30 Finally, the morphogens of the present invention may be evaluated for their therapeutic efficacy in causing a clinically significant improvement in a standard

marker of renal function when administered to a mammalian subject (e.g., a human patient) in, or at risk of, chronic renal failure. Such markers of renal function are well known in the medical literature and include, without being limited to, rates of increase in BUN levels, rates of increase in serum creatinine, static measurements of

5      BUN, static measurements of serum creatinine, glomerular filtration rates (GFR), ratios of BUN/creatinine, serum concentrations of sodium ( $\text{Na}^+$ ), urine/plasma ratios for creatinine, urine/plasma ratios for urea, urine osmolality, daily urine output, and the like (see, for example, Brenner and Lazarus (1994), in Harrison's Principles of Internal Medicine, 13th edition, Isselbacher et al., eds., McGraw Hill Text, New

10     York; Luke and Strom (1994), in Internal Medicine, 4th Edition, J.H. Stein, ed., Mosby-Year Book, Inc. St. Louis).

The morphogens contemplated herein can be expressed from intact or truncated genomic or cDNA or from synthetic DNAs in prokaryotic or eukaryotic host cells. The dimeric proteins can be isolated from the culture media and/or

15     refolded and dimerized in vitro to form biologically active compositions, Heterodimers can be formed in vitro by combining separate, distinct polypeptide chains. Alternatively, heterodimers can be formed in a single cell by co-expressing nucleic acids encoding separate, distinct polypeptide chains. See, for example, WO93/09229, or U.S. Pat. No. 5,411,941, for several exemplary recombinant

20     heterodimer protein production protocols. Currently preferred host cells include, without limitation, prokaryotes including *E. coli*, or eukaryotes including yeast (such as *S. cerevisiae*), insect cells, or any suitable mammalian host cells, such as CHO, COS or BSC cells. One of ordinary skill will appreciate that other host cells can be used to advantage. A detailed description of the morphogens useful in the methods,

25     compositions and devices of this invention, including how to make, use and test them for chondrogenic activity, are disclosed in numerous publications, including U.S. Pat. Nos. 5,266,683 and 5,011,691, the specifications of which are incorporated herein by reference.

As a general matter, methods of the present invention may be applied to the

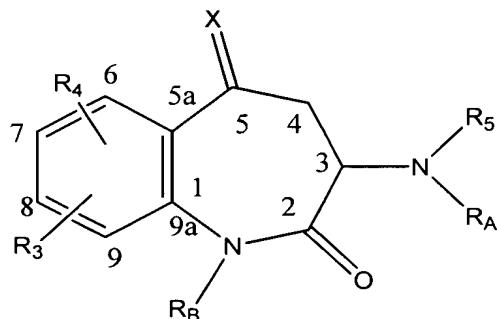
30     treatment of any mammalian subject at risk of or afflicted with a neural tissue insult or neuropathy. The invention is suitable for the treatment of any primate, preferably

a higher primate such as a human. In addition, however, the invention may be employed in the treatment of domesticated mammals which are maintained as human companions (*e.g.*, dogs, cats, horses), which have significant commercial value (*e.g.*, goats, pigs, sheep, cattle, sporting or draft animals), which have 5 significant scientific value (*e.g.*, captive or free specimens of endangered species, or inbred or engineered animal strains), or which otherwise have value.

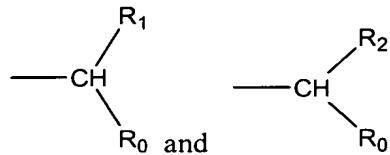
(ii) Inhibitors of ACE (Angiotensin-Converting Enzyme)

Angiotensin I-converting enzyme (EC 3.4.15.1), or kininase II, is a dipeptidyl carboxypeptidase that plays an important role in blood pressure regulation 10 and electrolyte balance by hydrolyzing angiotensin I into angiotensin II, a potent vasopressor, and aldosterone-stimulating peptide. The enzyme is also able to inactivate bradykinin, a potent vasodilator. The ACE gene encodes 2 isozymes. The somatic ACE isozyme is expressed in many tissues, including vascular endothelial cells, renal epithelial cells, and testicular Leydig cells, whereas the testicular or 15 germinal ACE isozyme is expressed only in sperm (Ramaraj et al., *J. Clin. Invest.* 102: 371-378, 1998).

The angiotensin converting enzyme (ACE) inhibitors of the present invention may include 3-amino-[1]benzazepin-2-one-1-alkanoic acids and derivatives, as disclosed in U.S. Patent Nos. 4,473,575 and 4,410,520 (the entire contents of which 20 are incorporated herein by reference), and characterized by formula (I) shown below:



wherein R<sub>A</sub> and R<sub>B</sub> are radicals of the formula:



respectively, in which:

R<sub>0</sub> is carboxy or a functionally modified carboxy;

5       R<sub>1</sub> is hydrogen, lower alkyl, amino(lower)alkyl, aryl, aryl(lower)alkyl,  
cycloalkyl, cycloalkyl(lower)alkyl, acylamino(lower) alkyl, mono- or di-  
(lower)alkylamino(lower)alkyl, lower alkylthio(lower)alkyl, carboxy(lower)alkyl,  
esterified carboxy(lower)alkyl, carbamoyl(lower)alkyl, etherified or acylated  
hydroxyl(lower)alkyl, aryloxy(-lower)alkyl, aryl-(thio-, sulfinyl-, or sulfonyl-)lower  
10      alkyl, aryl-N-(lower)alkylamino(lower)alkyl, or arylamino(lower)alkyl;

R<sub>2</sub> is hydrogen or lower alkyl;

R<sub>3</sub> and R<sub>4</sub>, each independently, represent hydrogen, lower alkyl, lower  
alkoxy, lower alkanoyloxy, hydroxyl, halogen, trifluoromethyl, or

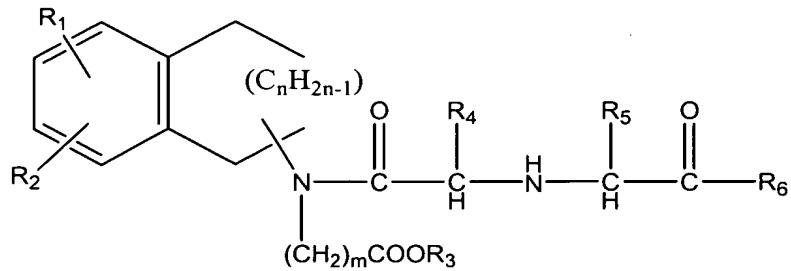
R<sub>3</sub> and R<sub>4</sub> taken together represent lower alkylenedioxy;

15      R<sub>5</sub> is hydrogen or lower alkyl, and

X represents oxo, two hydrogens, or one hydroxyl or acylated hydroxy  
together with one hydrogen; and wherein the carbocyclic ring may also be  
hexahydro or 6,7,8,9-tetrahydro; and salts and complexes thereof.

20      The functionally modified carboxyl group in the meaning of the symbol R<sub>0</sub>  
may be, for example, an esterified carboxyl group or a carbamoyl group optionally  
substituted on the nitrogen atom. More specifically one or both of R<sub>0</sub> represented  
by COR<sub>6</sub> in radical R<sub>A</sub> and represented by COR<sub>7</sub> in radical R<sub>B</sub> may independently  
represent carboxy, esterified carboxy, carbamoyl or substituted carbamoyl.

25      ACE inhibitors of the present invention may also include bicyclic  
compounds and their derivatives disclosed in U.S. Patent No. 4,385,051 (the entire  
contents of which are incorporated herein by reference), and represented by formula  
(II) shown below:



wherein R<sub>1</sub> and R<sub>2</sub> are the same or different and each represents hydrogen, hydroxyl or lower alkoxy;

5       R<sub>3</sub> is hydrogen or lower alkyl;

R<sub>4</sub> is hydrogen, lower alkyl, amino-lower-alkyl or acylamino-lower-alkyl;

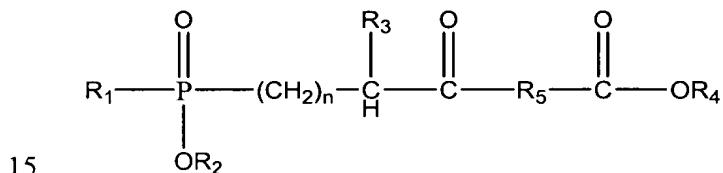
R<sub>5</sub> is hydrogen, lower alkyl or aralkyl which may be substituted;

R<sub>6</sub> is hydroxyl, lower alkoxy, amino or lower alkylamino;

and m and n each means 1 or 2, and salts thereof.

10

ACE inhibitors of the present invention may also include phosphinylalkanoyl substituted proline compounds disclosed in U.S. Patent No. 4,337,201 (the entire contents of which are incorporated herein by reference), and represented by formula (III) shown below:

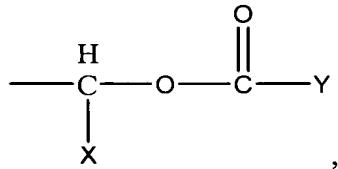


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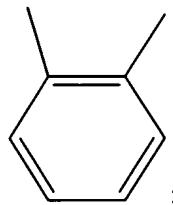
and salts thereof, wherein R<sub>1</sub> is alkyl, aryl, arylalkyl, cycloalkyl, or cycloalkyl(alkyl);

R<sub>2</sub> and R<sub>4</sub> each is independently hydrogen, alkyl, arylalkyl or

20

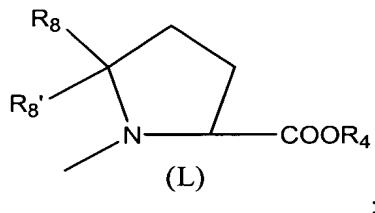
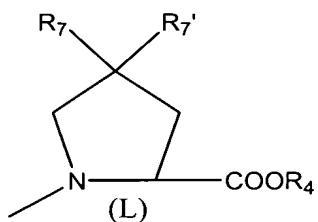
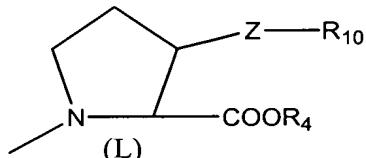
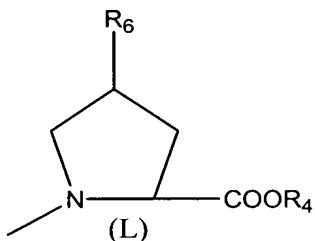


wherein X is hydrogen, alkyl, or phenyl and Y is hydrogen, alkyl, phenyl or alkoxy, or together X and Y are  $-(CH_2)_2-$ ,  $-(CH_2)_3-$ ,  $-CH=CH-$ , or



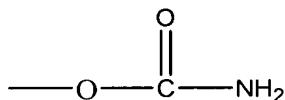
; R<sub>3</sub> is hydrogen or alkyl;

5 -R<sub>5</sub>-COOR<sub>4</sub> is



R<sub>6</sub> is hydrogen, hydroxyl, alkyl, halogen, azido, amino, cycloalkyl, aryl, arylalkyl, carbamoyloxy;

10



N,N-dialkylcarbamoyloxy, or -Z-R<sub>9</sub>;

15 R<sub>7</sub> and R<sub>7'</sub> are the same and each is halogen or -Z-R<sub>10</sub>, or R<sub>7</sub> and R<sub>7'</sub> together are =O, -O-(CH<sub>2</sub>)<sub>m</sub>-O- or -S-(CH<sub>2</sub>)<sub>m</sub>-S-;

R<sub>8</sub> is hydrogen and R<sub>8'</sub> is phenyl, 2-hydroxyphenyl or 4-hydroxyphenyl or R<sub>8</sub> and R<sub>8'</sub> together are =O;

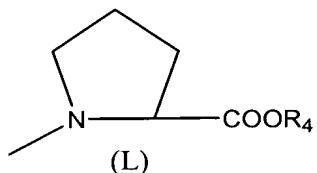
R<sub>9</sub> is alkyl, aryl, arylalkyl, 1- or 2-naphthyl, or biphenyl;

R<sub>10</sub> is alkyl, aryl or arylalkyl;

Z is oxygen or sulfur;

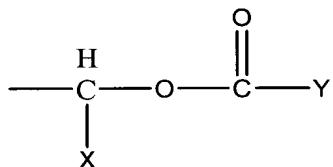
n is 0 or 1; and

5 m is 1 or 2; with the proviso that if -R<sub>5</sub>-COOR<sub>4</sub> is

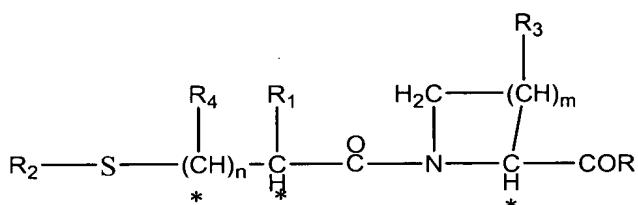


at least one of R<sub>2</sub> and R<sub>4</sub> is

10



ACE inhibitors of the present invention may also include azetidine-2-carboxylic acid derivative compounds disclosed in U.S. Patent No. 4,046,889 (the 15 entire contents of which are incorporated herein by reference), and represented by formula (IV) shown below:



wherein R is hydroxy, NH<sub>2</sub> or lower alkoxy;

R<sub>1</sub> and R<sub>4</sub> each is hydrogen, lower alkyl or phenyl-lower alkyl;

20 R<sub>2</sub> is hydrogen or R<sub>5</sub>-CO;

R<sub>3</sub> is hydrogen, hydroxy or lower alkyl;

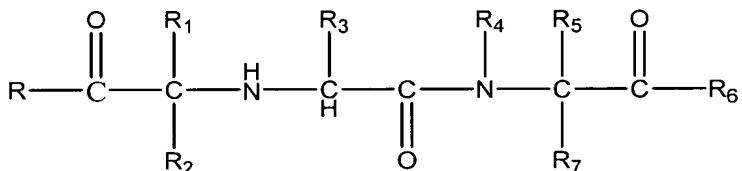
$R_5$  is lower alkyl, phenyl or phenyl-lower alkyl;

$m$  is 1 to 3;

$n$  is 0 to 2, and the asterisks indicate asymmetric carbon atoms. The carbon in the acyclic side chain is asymmetric when  $R_1$  is other than hydrogen.

5

ACE inhibitors of the present invention may also include carboxyalkyl dipeptide compounds and derivatives thereof, disclosed in U.S. Patent No. 4,374,829 (the entire contents of which are incorporated herein by reference), and represented by formula (V) shown below:



10

wherein  $R$  and  $R_6$  are the same or different and are hydroxy, lower alkoxy, lower alkenoxy, dilower alkylamino lower alkoxy (dimethylaminoethoxy), acylamino lower alkoxy (acetylaminoethoxy), acyloxy lower alkoxy (pivaloyloxymethoxy), aryloxy such as phenoxy, aryl(lower)alkoxy such as benzyloxy; substituted aryloxy or substituted aryl(lower)alkoxy wherein the substituent is methyl, halo or methoxy, amino, lower alkylamino, di(lower)alkylamino, hydroxyamino, or aryl(lower)alkylamino such as benzylamino;

$R_1$  is hydrogen, alkyl of from 1 to 20 carbon atoms which include branched and cyclic and unsaturated (such as allyl) alkyl groups, substituted lower alkyl wherein the substituent can be halo, hydroxy, lower alkoxy, aryloxy such as phenoxy, amino, dilower alkylamino, acylamino, such as acetamido and benzamidoaryl amino, guanidino, imidazolyl, indolyl, mercapto, lower alkylthio, arylthio such as phenylthio, carboxy or carboxyamido, carbolower alkoxy, aryl such as phenyl or naphthyl, substituted aryl such as phenyl wherein the substituent is lower alkyl, lower alkoxy or halo, aryl lower alkyl, aryl lower alkenyl, heteroaryl lower alkyl or heteroaryl lower alkenyl such as benzyl, styryl or indolyl ethyl, substituted aryl lower alkyl, substituted aryl lower alkenyl, substituted heteroaryl lower alkyl, or substituted heteroaryl lower alkenyl, wherein the substituent(s) is

halo, dihalo, lower alkyl, hydroxy, lower alkoxy, amino, aminomethyl, acylamino (acetylamino or benzoylamino) dilower alkylamino, lower alkylamino, carboxyl, halolower alkyl, cyano or sulfonamide, aryl lower alkyl or heteroaryllowrealkyl substituted on the alkyl portion by amino or acylamino (acetylamino or

5     benzoylamino);

R<sub>2</sub> and R<sub>7</sub> are the same or different and are hydrogen or lower alkyl;

R<sub>3</sub> is hydrogen, lower alkyl, phenyl lower alkyl, aminomethyl phenyl lower alkyl, hydroxy phenyl lower alkyl, hydroxy lower alkyl, acylamino lower alkyl (such as benzoylamino lower alkyl, acetylamino lower alkyl) amino lower alkyl,

10    dimethylamino lower alkyl, halo lower alkyl, guanidino lower alkyl, imidazolyl lower alkyl, indolyl lower alkyl, mercapto lower alkyl, lower alkyl thio lower alkyl;

R<sub>4</sub> is hydrogen or lower alkyl;

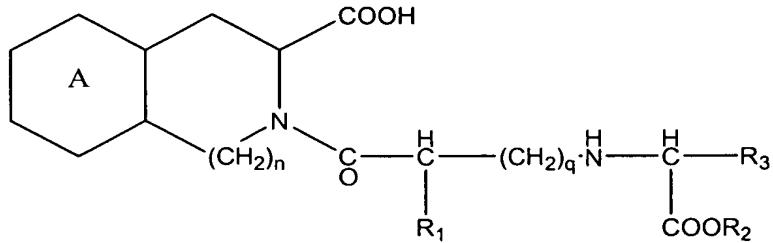
R<sub>5</sub> is hydrogen, lower alkyl, phenyl, phenyl lower alkyl, hydroxyl phenyl lower alkyl, hydroxyl lower alkyl, amino lower alkyl, guanidino lower alkyl,

15    imidazolyl lower alkyl, indolyl lower alkyl, imidazolyl lower alkyl, indolyl lower alkyl, mercapto lower alkyl or lower alkyl thio lower alkyl;

R<sub>4</sub> and R<sub>5</sub> may be connected together to form an alkylene bridge of from 2 to 4 carbon atoms, an alkylene bridge of form 2 to 3 carbon atoms and one sulfur atom, an alkylene bridge of from 3 to 4 carbon atoms containing a double bond or an

20    alkylene bridge as above substituted with hydroxyl, lower alkoxy, lower alkyl or dilower alkyl.

ACE inhibitors of the present invention may also include substituted iminodiacid compounds as disclosed in U.S. Patent No. 4,508,729 (the entire contents of which are incorporated herein by reference), and represented by formula (VI) shown below:

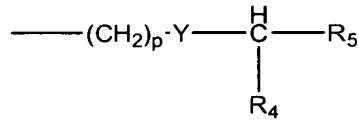


wherein the ring A is saturated and n=0 or 1, or the ring A is a benzene ring and n=1,

R<sub>1</sub> represents a lower alkyl group having from 1 to 4 carbon atoms which can  
5 carry an amino group,

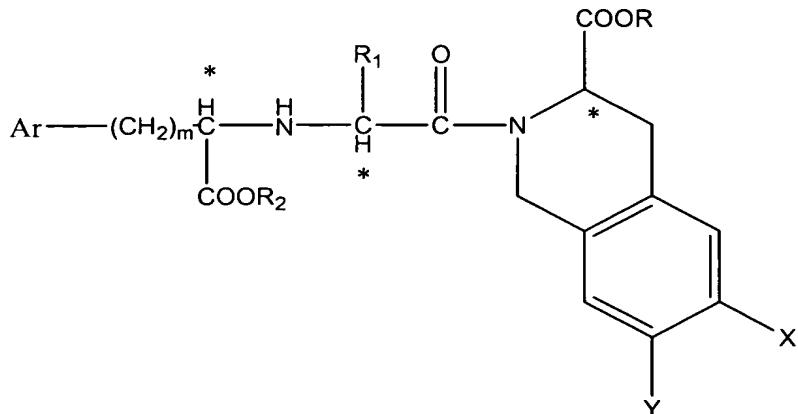
R<sub>2</sub> represents a hydrogen atom or an alkyl group having from 1 to 4 carbon atoms,

R<sub>3</sub> represents a straight or branched alkyl group, a mono- or  
dicycloalkylalkyl or phenylalkyl group having no more than a total of 9 carbon  
10 atoms, or a substituted alkyl group of the formula:



with R<sub>4</sub> = H, a lower alkyl (C<sub>1</sub> to C<sub>4</sub>) or a cycloalkyl (C<sub>3</sub> to C<sub>6</sub>) group,  
15 R<sub>5</sub>=H, a lower alkyl (C<sub>1</sub> to C<sub>4</sub>), a cycloalkyl (C<sub>3</sub> to C<sub>6</sub>) or an alkoxy carbonyl group,  
Y=S or >N-Q where Q=H, or an acetyl or benzyloxycarbonyl group, and p=1 or 2, and q=0 or 1.

20 The ACE inhibitor compounds of the present invention may also include substituted acyl derivatives of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid compounds disclosed in U.S. Patent No. 4,344,949 (the entire contents of which are incorporated herein by reference), and having formula (VII) as shown below:



where R is hydrogen, lower alkyl or aralkyl;

R<sub>1</sub> is hydrogen, lower alkyl, or benzyl;

R<sub>2</sub> is hydrogen or lower alkyl, and Ar is phenyl or phenyl substituted with 1

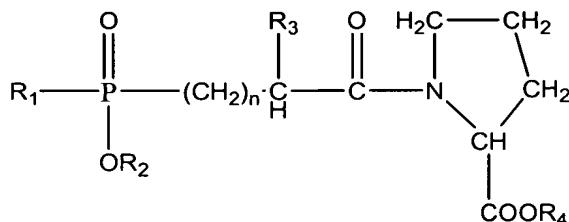
- 5 or 2 substituents selected from the group consisting of fluorine, chlorine, bromine, lower alkyl, lower alkoxy, hydroxy or amino;

X and Y are independently hydrogen, lower alkyl, lower alkoxy, lower alkylthio, lower alkylsufinyl, lower alkylsulfonyl, hydroxy, or X and Y together are methylenedioxy;

10 m is 0 to 3;

and pharmaceutically acceptable salts thereof.

The ACE inhibitors may also include phosphinylalkanoyl proline compounds disclosed in U.S. 4,168,267 (the entire content of which is incorporated herein by reference), which have formula (VIII) as shown below:



wherein R<sub>1</sub> is lower alkyl, phenyl or phenyl-lower alkyl;

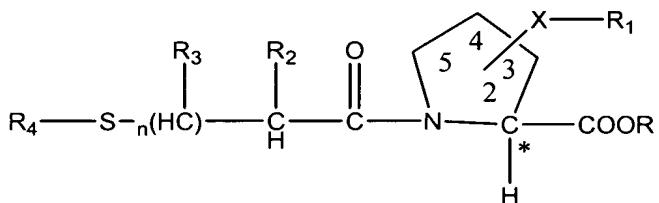
R<sub>2</sub> is hydrogen, phenyl-lower alkyl or a metal ion;

R<sub>3</sub> is hydrogen or lower alkyl;

R<sub>4</sub> is hydrogen, lower alkyl, phenyl-lower alkyl or a metal ion; and

n is 0 or 1.

5        The ACE inhibitor compounds of the present invention may also include ether and thioether mercaptoacyl proline compounds disclosed in U.S. Patent No. 4,316,906 (the entire content of which is incorporated herein by reference), and having formula (IX) as shown below:



10        wherein the group X-R<sub>1</sub> is located at the 3- or 4-position in the ring;

X is oxygen or sulfur;

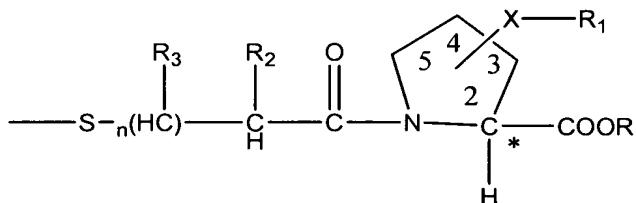
R is hydrogen or lower alkyl;

15        R<sub>1</sub> is lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, 1- or 2-adamantyl, aryl, substituted aryl, phenyl-lower alkylene or substituted phenyl-lower alkylene.

R<sub>2</sub> and R<sub>3</sub> are independently selected from hydrogen, lower alkyl, and trifluoromethyl;

R<sub>4</sub> is hydrogen, R<sub>5</sub> - CO -, or

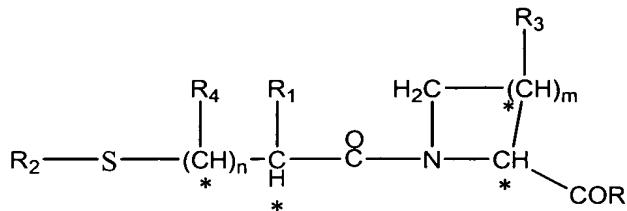
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R<sub>5</sub> is lower alkyl, phenyl, phenyl-lower alkylene; substituted phenyl, or substituted phenyl-lower alkylene;

n is 0, 1 or 2; and salts thereof.

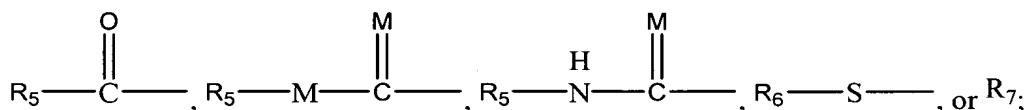
- The ACE inhibitor compounds of the present invention also may include proline derivatives and related compounds disclosed in U.S. Patent No. 4,105,776  
5 (the entire contents of which are incorporated herein by reference), which have the general formula (X) as shown below:



wherein R is hydroxy, NH<sub>2</sub> or lower alkoxy;

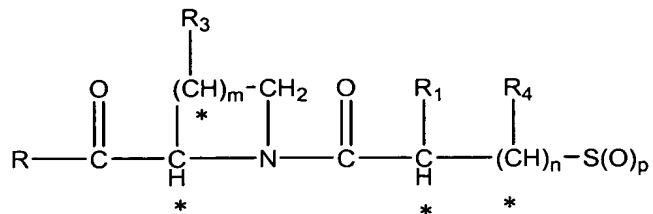
- 10 R<sub>1</sub> and R<sub>4</sub> each is hydrogen, lower alkyl, phenyl or phenyl-lower alkyl;  
R<sub>2</sub> is hydrogen, lower alkyl, phenyl, substituted phenyl wherein the phenyl substituent is halo, lower alkyl or lower alkoxy, phenyl-lower alkyl, diphenyl-lower alkyl, triphenyl-lower alkyl, lower alkylthiomethyl, phenyl-lower alkythiomethyl, lower alkanoyl-amidomethyl,

15



R<sub>3</sub> is hydrogen, hydroxyl or lower alkyl;

- 15 R<sub>5</sub> is lower alkyl, phenyl or phenyl-lower alkyl;  
R<sub>6</sub> is lower alkyl, phenyl, substituted phenyl, (wherein the phenyl substituent is halo, lower alkyl or lower alkoxy), hydroxyl-lower alkyl or amino(carboxy)lower alkyl;



M is O or S;

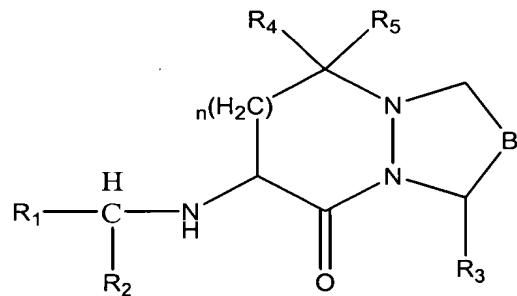
*m* is 1 to 3; and

$n$  and  $p$  each is 0 to 2.

- 5 The asterisks indicate asymmetric carbon atoms. Each of the carbons bearing a substituent  $R_1$ ,  $R_3$  and  $R_4$  is asymmetric when that substituent is other than hydrogen.

The ACE inhibitors of the present invention may also include bicyclic

- 10 pyridazo [1,2-A][1,2] diazepine compounds disclosed in U.S. Patent No. 4,512,924  
(the entire contents of which are incorporated herein by reference), having the  
general formula (XI) as shown below:



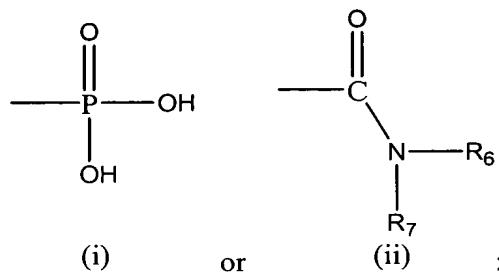
- 15 wherein B represent a methylene ( $-\text{CH}_2-$ ) , ethylene ( $-\text{CH}_2-\text{CH}_2-$ ) or  
vinylene ( $-\text{CH}=\text{CH}-$ ) group;

R<sub>1</sub> represents a hydrogen atom or an alkyl, aralkyl, amino-alkyl,  
monoalkylamino-alkyl, dialkylaminoalkyl, acylamino-alkyl, phthalimido-alkyl,  
alkoxycarbonylamino-alkyl, aryloxycarbonylamino-alkyl, aralkoxycarbonlyamino-  
alkyl, alkylaminocarbonylaminooalkyl, arylaminocarbonylaminoo-alkyl,

aralkylaminocarbonylamino-alkyl, alkylsulphonylamino-alkyl or arylsulphonylamino-alkyl group;

R<sub>2</sub> represents a carboxyl, alkoxy carbonyl or aralkoxy carbonyl group or a group of the formula:

5



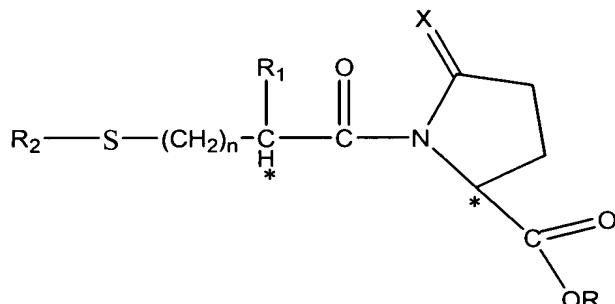
(i) or (ii);

R<sub>3</sub> represents a carboxyl, alkoxy carbonyl or aralkoxy carbonyl group;  
R<sub>4</sub> and R<sub>5</sub> each represent a hydrogen atom or R<sub>4</sub> and R<sub>5</sub> together represent an oxo group;

10 R<sub>6</sub> and R<sub>7</sub> each represent a hydrogen atom or an alkyl or aralkyl group or R<sub>6</sub> and R<sub>7</sub> together with the nitrogen atom to which they are attached represent a saturated 5 membered or 6-membered heteromonocyclic ring which may contain a further nitrogen atom or an oxygen or sulphur atom, and n stands for zero, 1 or 2, and pharmaceutically acceptable salts thereof.

15

The ACE inhibitors may also include pyroglutamic acid derivatives as disclosed in U.S. Patent No. 4,234,489 (the entire contents of which are incorporated herein by reference), having the formula (XII) as shown below:

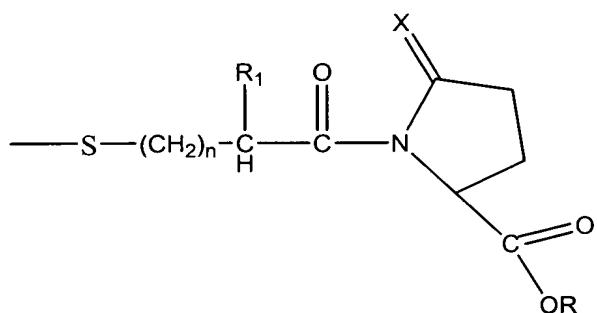


20 and salts thereof, wherein

R is hydrogen, alkyl or diphenylmethyl;

R<sub>1</sub> is hydrogen, alkyl or trifluoromethyl;

R<sub>2</sub> is hydrogen,



5

R<sub>3</sub> is hydrogen, alkyl, phenyl, or phenylalkyl;

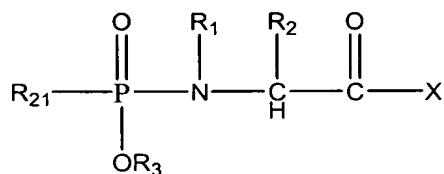
X is oxygen or sulfur; and

n is 0 or 1.

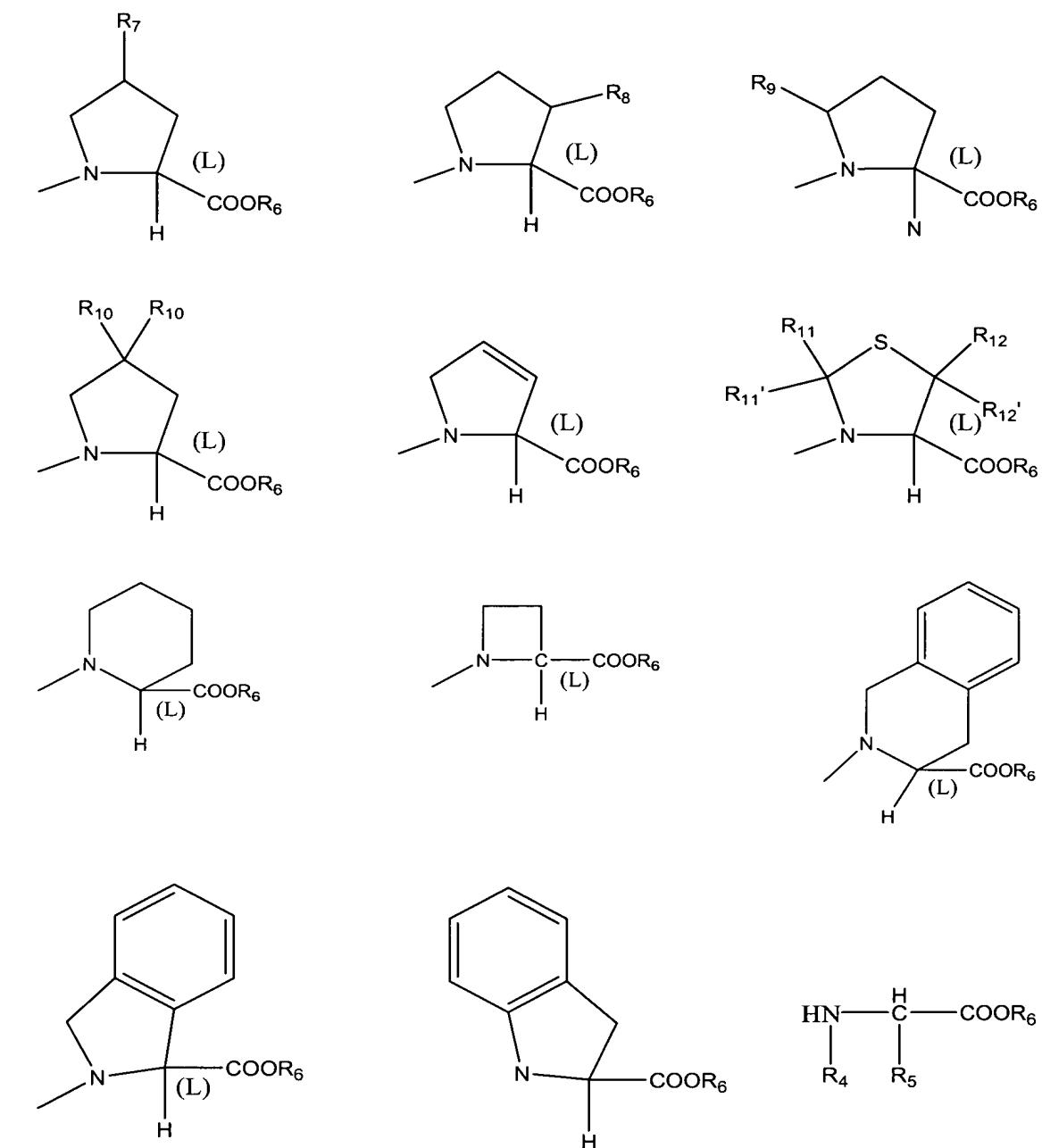
10

The ACE inhibitor compounds may also include the phosphonamidate substituted amino or imino acids and salts thereof of U.S. Patent No. 4,432,971 (the entire contents of which are incorporated herein by reference), and of the formula (XIII) as shown below:

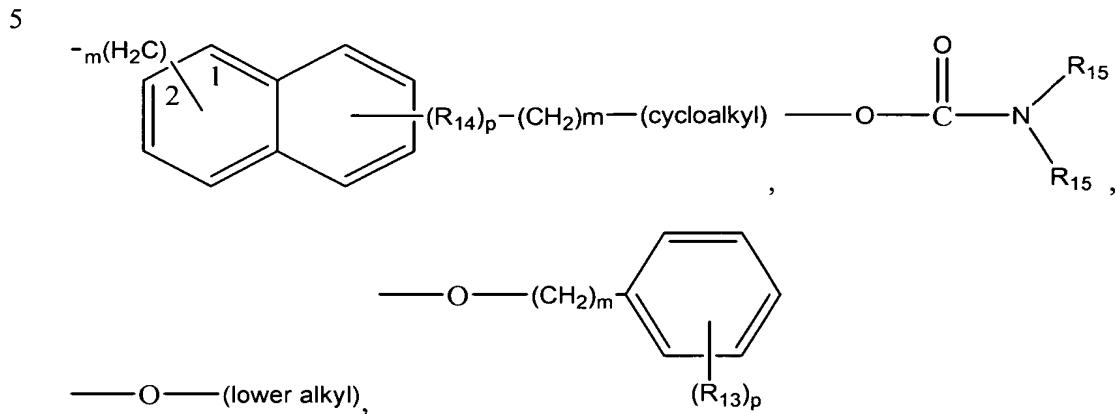
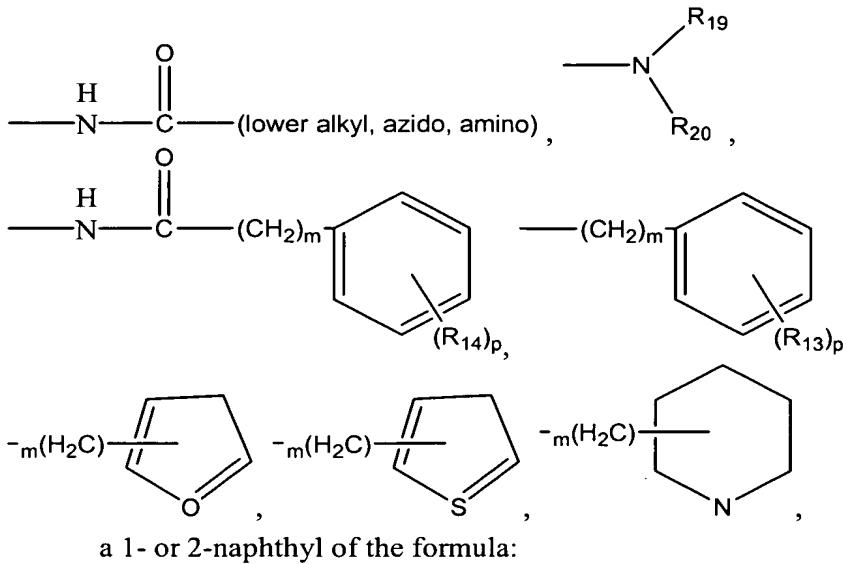
15



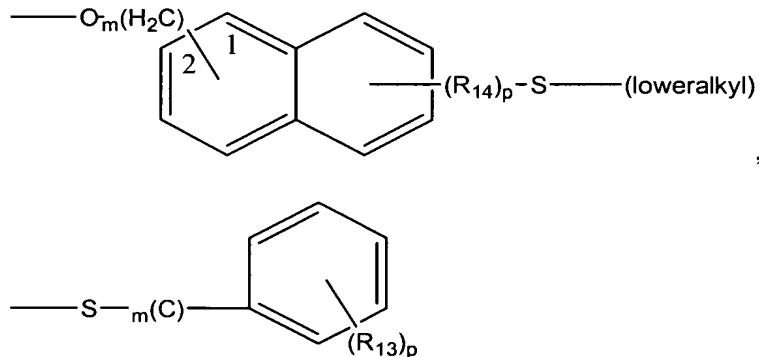
wherein X is an imino or amino acid of the formula:



R<sub>7</sub> is hydrogen, lower alkyl, halogen, keto, hydroxyl,

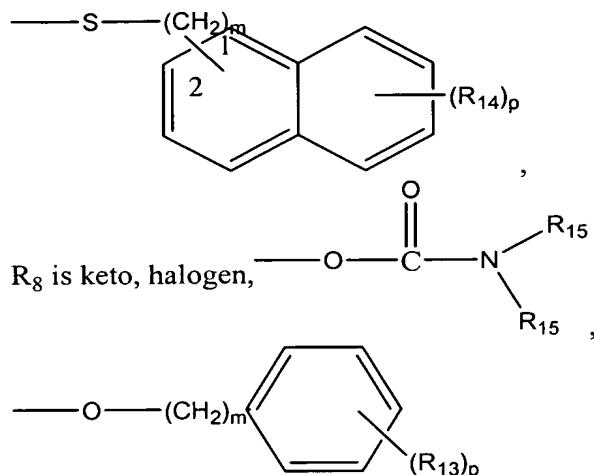


10        a 1- or 2- naphthyoxy of the formula:

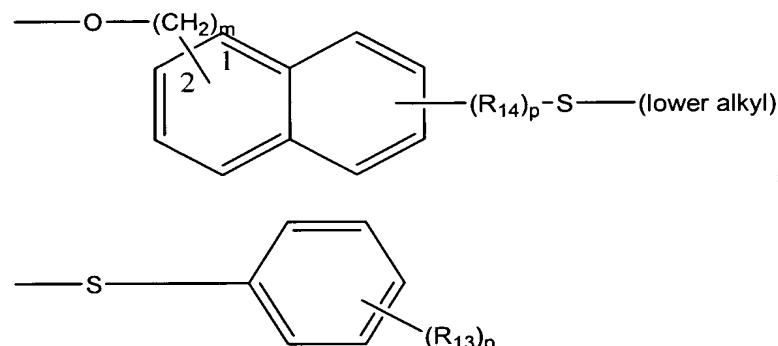


15

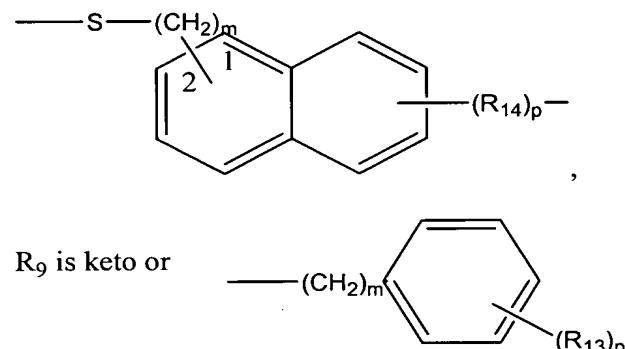
or a 1- or 2-naphthylthio of the formula:



5           -O—lower alkyl, a 1- or 2-naphthoxy of the formula:

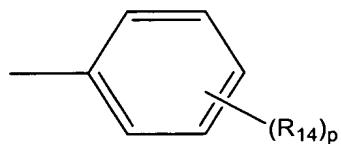


10           or a 1- or 2-naphthylthio of the formula:



15           R<sub>10</sub> is halogen or —Y—R<sub>16</sub>,

R<sub>11</sub>, R<sub>11</sub>, R<sub>12</sub> and R<sub>12</sub> are independently selected from hydrogen and lower alkyl or R<sub>11</sub>, R<sub>12</sub> and R<sub>12</sub> are hydrogen and R<sub>11</sub> is



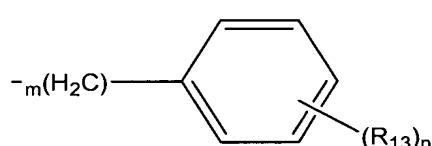
- R<sub>13</sub> is hydrogen, lower alkyl of 1 to 4 carbons, lower alkythio of 1 to 4  
5 carbons, chloro, bromo, fluoro, trifluoromethyl, hydroxyl, phenyl, phenoxy,  
phenylthio, or phenylmethyl.
- R<sub>14</sub> is hydrogen, lower alkyl of 1 to 4 carbons, lower alkoxy of 1 to 4  
carbons, lower alkylthio of 1 to 4 carbons, chloro, bromo, fluoro, trifluoromethyl, or  
hydroxy.

10 m is zero, one two or three;  
p is one, two or three provided that p is more than one only if R<sub>13</sub> or R<sub>14</sub> is  
hydrogen, methyl, methoxy, chloro, or fluoro;

R<sub>15</sub> is hydrogen or lower alkyl of 1 to 4 carbons;

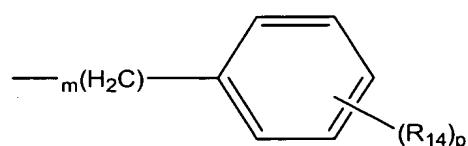
Y is oxygen or sulfur;

15 R<sub>16</sub> is lower alkyl of 1 to 4 carbons;

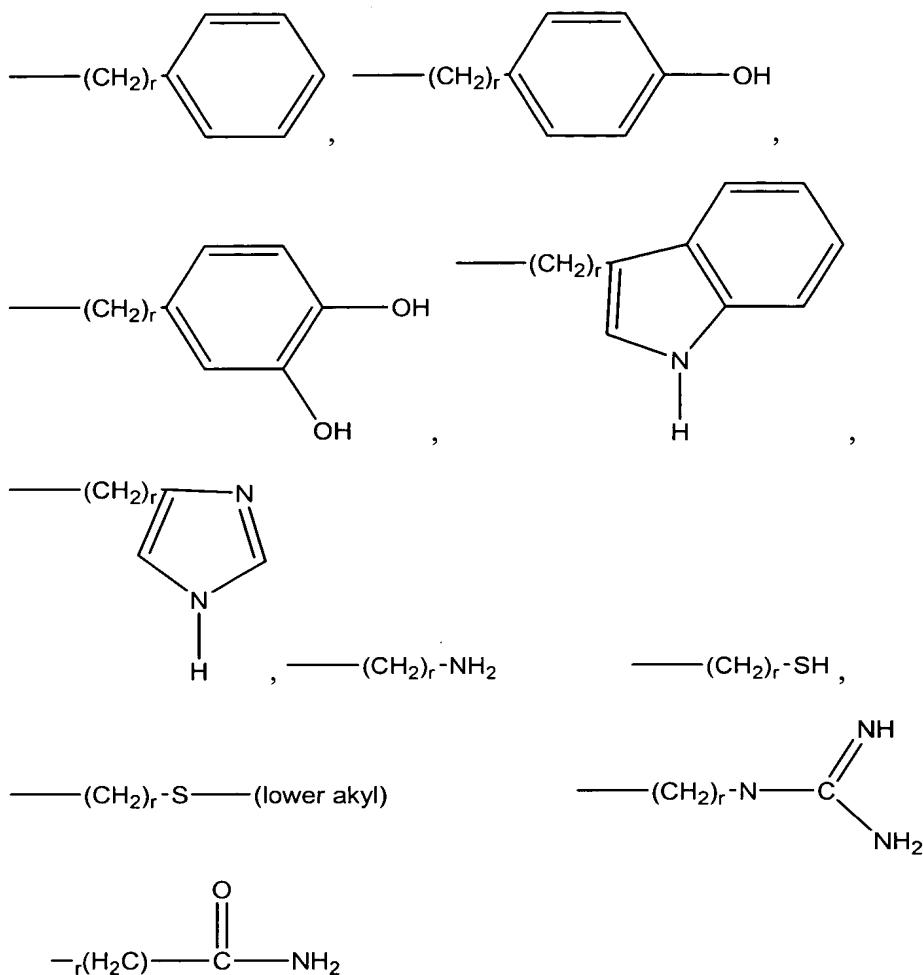


or the R<sub>16</sub> groups join to complete an unsubstituted 5- or 6-membered ring or  
said ring in which one or more of the carbons has a lower alkyl of 1 to 4 carbons or a  
di(lower alkyl of 1 to 4 carbons) substituent.

20 R<sub>4</sub> is hydrogen, lower alkyl,  $-(CH_2)m$ —cycloalkyl,  
or



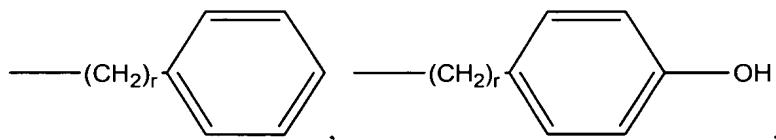
R<sub>5</sub> is hydrogen, lower alkyl,



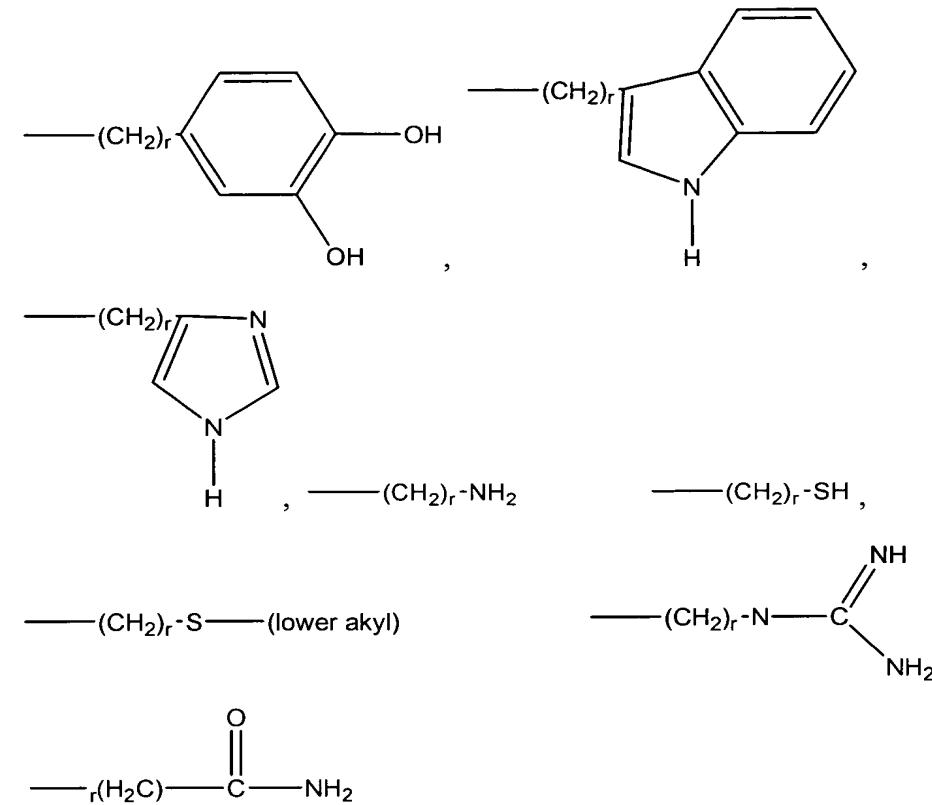
10 r is an integer from 1 to 4.

$R_1$  is hydrogen, lower alkyl, or cycloalkyl.

R<sub>2</sub> is hydrogen, lower alkyl, halo substituted lower alkyl,



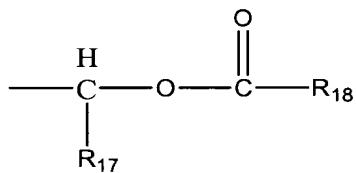
15



5 or  $R_1$  and  $R_2$  taken together are  $-(CH_2)_n-$  wherein  $n$  is an integer from 2 to

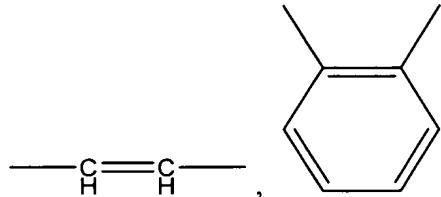
4.

10  $R_3$  and  $R_6$  are independently selected from hydrogen, lower alkyl, benzyl, benzhydryl, or



15 wherein R<sub>17</sub> is hydrogen, lower alkyl, cycloalkyl, or phenyl, and R<sub>18</sub> is hydrogen, lower alkyl, lower alkoxy, phenyl, or R<sub>17</sub> and R<sub>18</sub> taken together are

$-(CH_2)_2-$        $-(CH_2)_3-$ ,

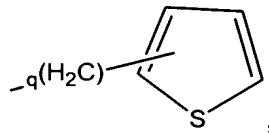
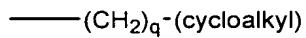
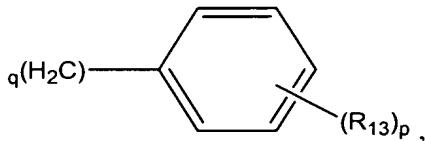


R<sub>19</sub> is lower alkyl, benzyl, or phenethyl;

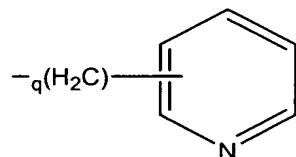
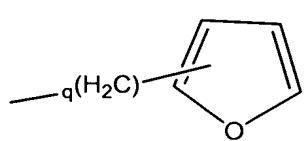
R<sub>20</sub> is hydrogen, lower alkyl, benzyl or phenethyl;

R<sub>21</sub> is alkyl of 1 to 10 carbons;

5



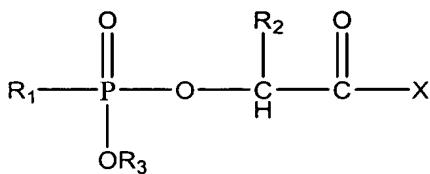
10



wherein q is zero or an integer from 1 to 7, s is an integer from 1 to 8, and R<sub>13</sub>, and p is as defined above.

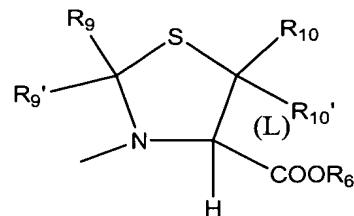
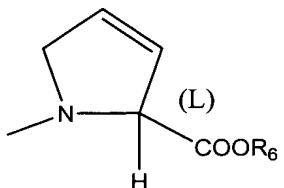
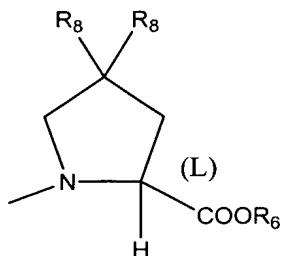
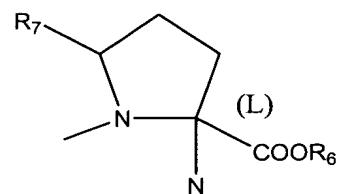
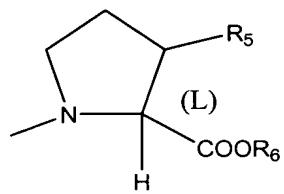
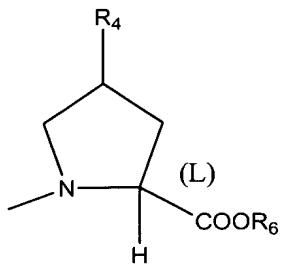
15

The ACE inhibitor compounds of the present invention may also include the phosphonate substituted amino or imino acids and salts thereof, as disclosed in U.S. Patent No. 4,452,790 (the entire contents of which are incorporated herein by reference), of the general formula (XIV) as shown below:

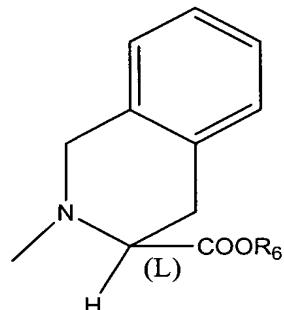
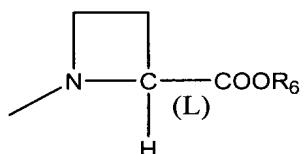
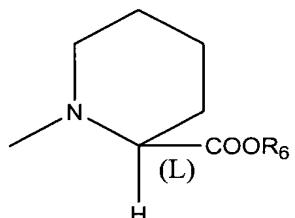


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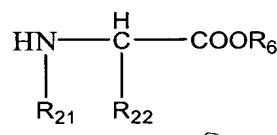
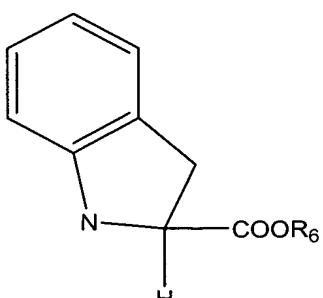
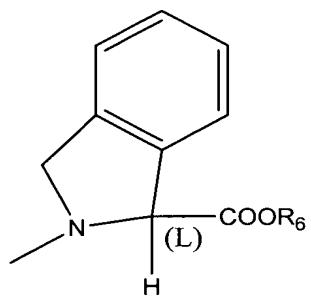
X is an imino or amino acid of the formula:



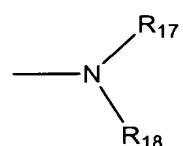
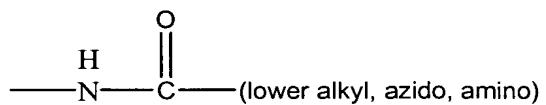
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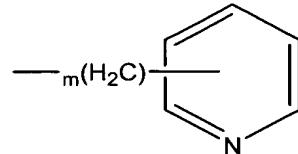
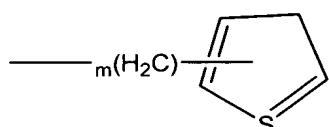
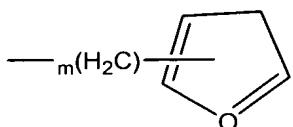
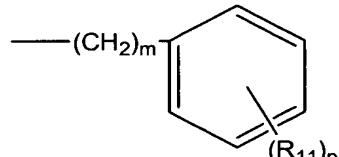
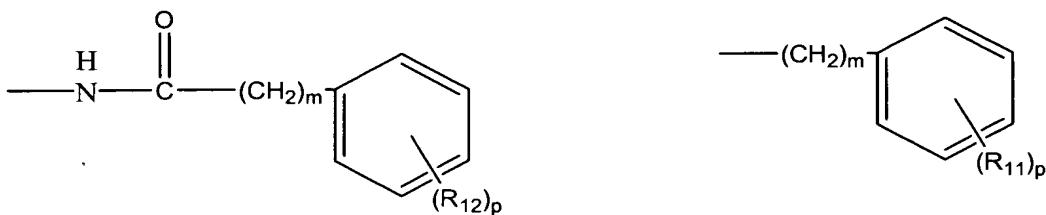


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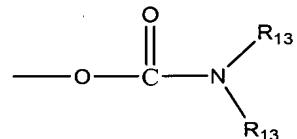
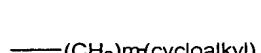
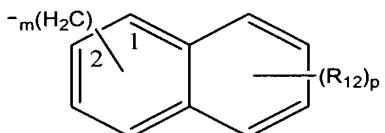
R4 is hydrogen, lower alkyl, halogen, keto, hydroxy,



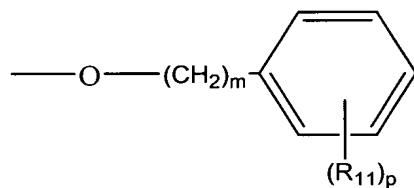
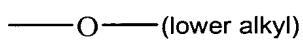


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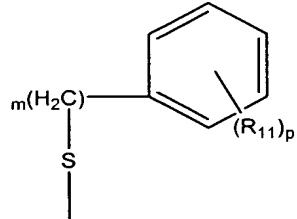
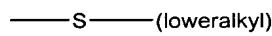
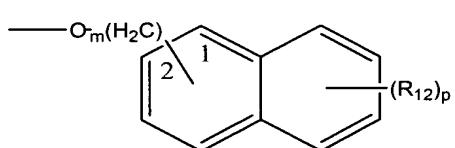
a 1- or 2-naphthyl of the formula:



10

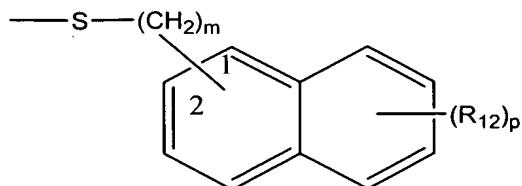


a 1- or 2-naphthyoxy of the formula:

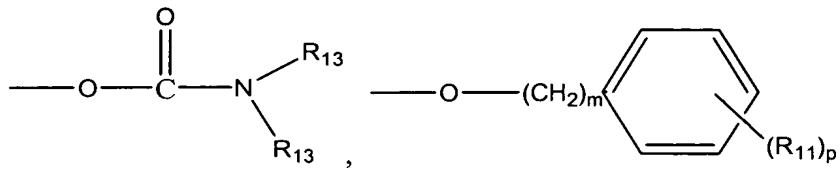


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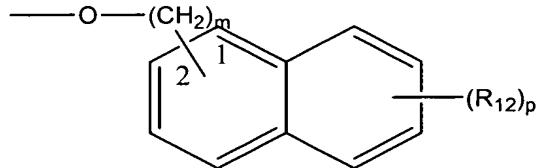
or a 1- or 2-naphthylthio of the formula:



R<sub>5</sub> is keto, halogen,

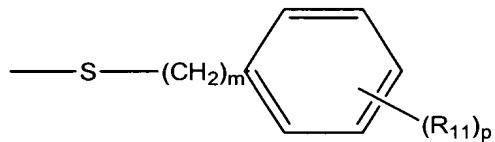


5        —O—lower alkyl, a 1- or 2-naphthyoxy of the formula:



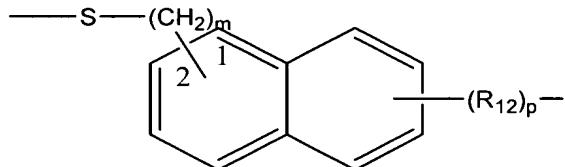
—S—lower alkyl,

10



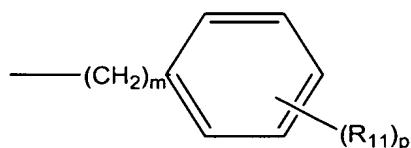
or a 1- or 2-naphthylthio of the formula:

15



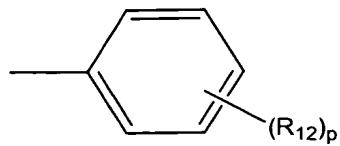
R<sub>7</sub> is keto or

20



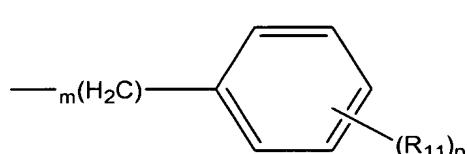
each R<sub>8</sub> is independently halogen or —Y—R<sub>14</sub>;

R<sub>9</sub>, R<sub>9</sub>, R<sub>10</sub> are independently selected from hydrogen and lower alkyl or R<sub>9</sub>, R<sub>10</sub> and R<sub>10</sub> are hydrogen and R<sub>9</sub> is



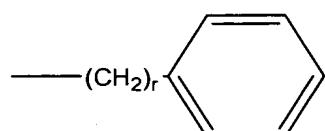
- R<sub>11</sub> is hydrogen, lower alkyl of 1 to 4 carbons, lower alkoxy of 1 to 4  
5 carbons, lower alkythio of 1 to 4 carbons, chloro, bromo, fluoro, trifluormethyl,  
hydroxyl, phenyl, phenoxy, phenylthio, or phenylmethyl;
- R<sub>12</sub> is hydrogen, lower alkyl of 1 to 4 carbons, lower alkoxy of 1 to 4  
carbons, lower alkythio of 1 to 4 carbons, chloro, bromo, fluoro, trifluoromethyl, or  
hydroxy;
- 10 m is zero, one two or three;
- p is one, two or three provided that p is more than one only if R<sub>11</sub> or R<sub>12</sub> is  
hydrogen, methyl, methoxy, chloro, fluoro;
- R<sub>13</sub> is hydrogen or lower alkyl of 1 to 4 carbons;
- Y is oxygen or sulfur;

15 R<sub>14</sub> is lower alkyl of 1 to 4 carbons;

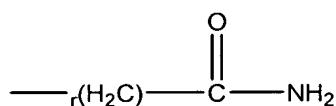
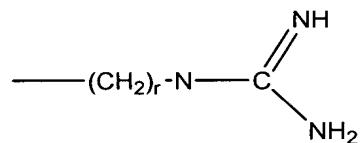
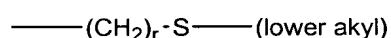
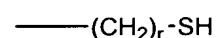
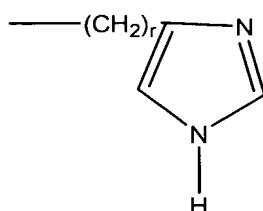
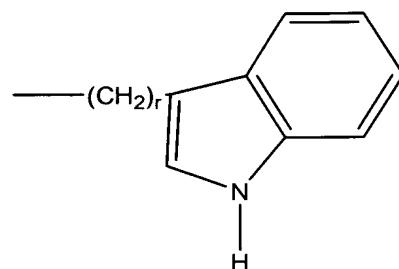
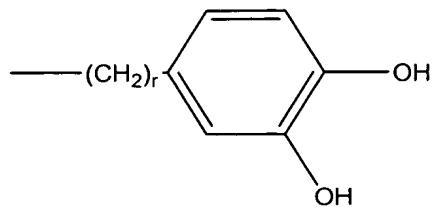
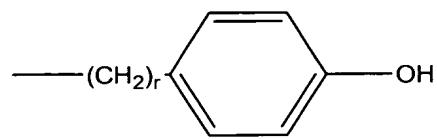
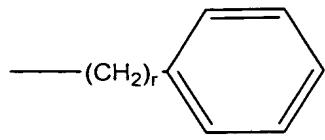


or the R<sub>14</sub> groups join to complete an unsubstituted 5- or 6-membered ring or  
said ring in which one or more of the carbons has a lower alkyl of 1 to 4 carbons or a  
20 di(lower alkyl of 1 to 4 carbons) substituent;

R<sub>21</sub> is hydrogen, lower alkyl, cycloalkyl, phenyl, or



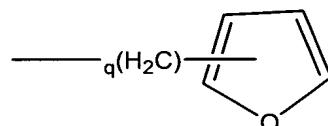
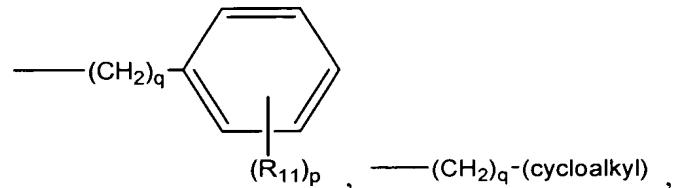
R<sub>22</sub> is hydrogen, lower alkyl,

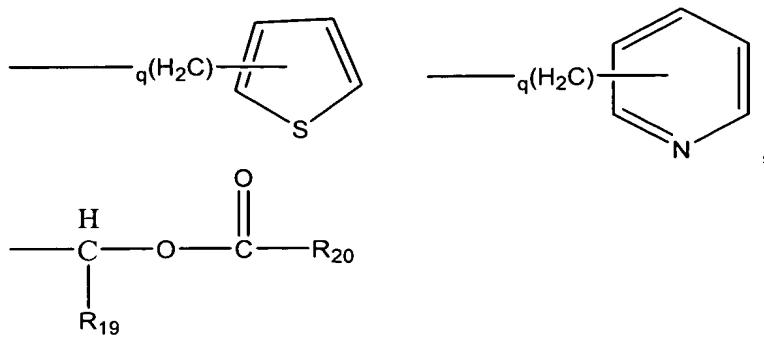


5

r is an integer from 1 to 4;

R<sub>1</sub> is alkyl of 1 to 10 carbons, aminoalkyl, haloalkyl;

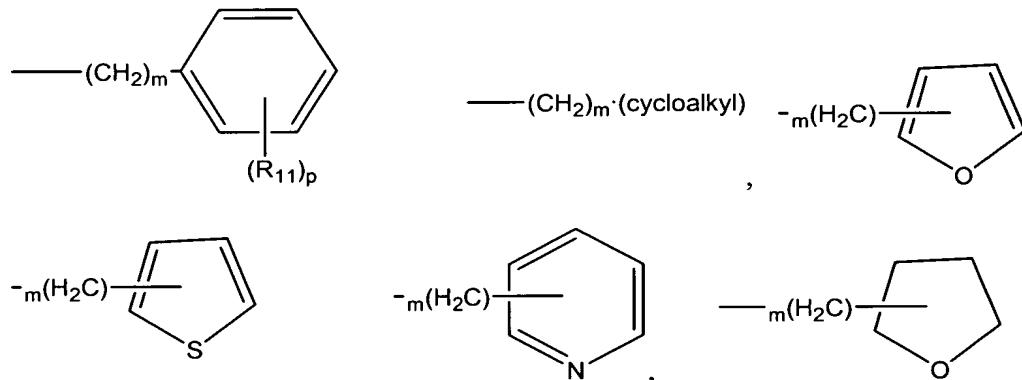




wherein  $q$  is zero or an integer from 1 to 7 and  $R_{12}$  and  $p$  are defined as above;

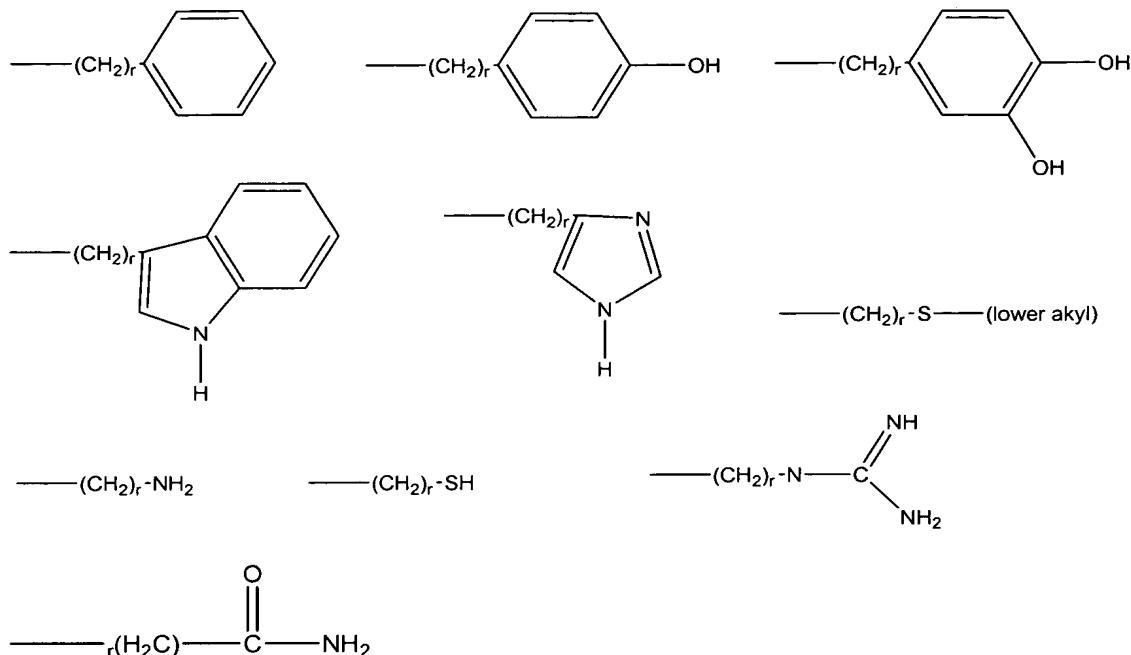
5         $R_{19}$  and  $R_{20}$  are independently selected from hydrogen, lower alkyl, halo substituted lower alkyl;

10



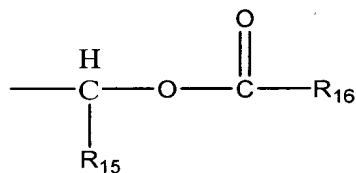
wherein  $m$ ,  $R_{11}$ , and  $p$  are as defined above;

$R_2$  is hydrogen, lower alkyl, halo substituted lower alkyl;

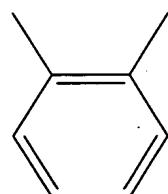


5 wherein r is defined above.

R<sub>3</sub> and R<sub>6</sub> are independently selected from hydrogen, lower alkyl, benzyl, alkali metal such as Li, Na or K, benzhydryl, or



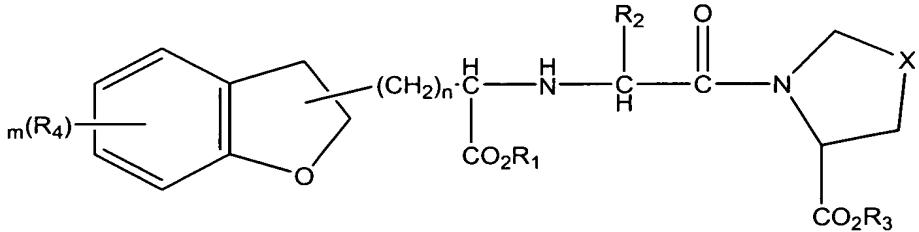
wherein R<sub>15</sub> is hydrogen, lower alkyl, cycloalkyl, or phenyl, and R<sub>16</sub> is  
10 hydrogen, lower alkyl, lower alkoxy, phenyl, or R<sub>15</sub> and R<sub>16</sub> taken together are –  
(CH<sub>2</sub>)<sub>2</sub>--, --(CH<sub>2</sub>)<sub>3</sub>--, --CH=CH--, or



R<sub>17</sub> is lower alkyl, benzyl, or phenethyl;

R<sub>18</sub> is hydrogen, lower alkyl, benzyl or phenethyl.

The ACE inhibitor compounds may also include the compounds disclosed in EP Patent No. 0060668 (the entire contents of which are incorporated herein by reference), having formula (XV) as shown below:



5 or a pharmaceutically acceptable salt thereof, wherein

m is 0 to 3;

n is 1 to 5;

R<sub>1</sub> is hydrogen or C<sub>1-6</sub>alkyl;

R<sub>2</sub> is hydrogen, C<sub>1-4</sub>alkyl, -(CH<sub>2</sub>)p-NH<sub>2</sub>

10 wherein p is 1 to 4, or -NHCOR<sub>5</sub> wherein R<sub>5</sub> is C<sub>1-4</sub>alkyl;

R<sub>3</sub> is hydrogen or C<sub>1-6</sub>alkyl;

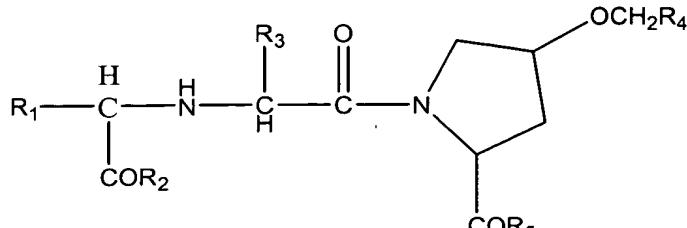
R<sub>4</sub> is C<sub>1-4</sub>alkyl, C<sub>1-4</sub>alkoxy, halogen or CF<sub>3</sub>; and

X is CH<sub>2</sub> or S;

15 The dihydrobenzofuran moiety may be bonded to the rest of the structure at the 2- or 3-position, preferably, the 2-position;

Preferably X is CH<sub>2</sub>.

20 The ACE inhibitor compounds may also be compounds as disclosed in EP Patent No. 0080822 (the entire contents of which are incorporated herein by reference), having formula (XVI) as shown below:



or a pharmaceutically acceptable salt thereof,

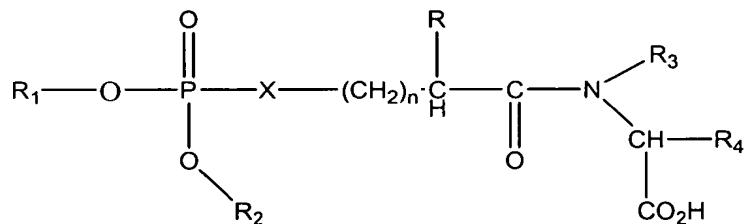
wherein R<sub>1</sub> is C<sub>1-5</sub> alkyl optionally substituted by NHR<sub>6</sub>, (wherein R<sub>6</sub> is hydrogen or C<sub>1-5</sub> alkylcarbonyl) or by phenyl or naphtyl optionally substituted by halogen, C<sub>1-5</sub> alkyl or C<sub>1-5</sub> alkoxy or by dihydrobenzofuran-2-yl, optionally substituted in the benzo moiety by C<sub>1-5</sub> alkyl, C<sub>1-5</sub> alkoxy, halogen or trifluoromethyl;

R<sub>2</sub> and R<sub>5</sub> are the same or different and each is hydroxyl, C<sub>1-5</sub> alkoxy, C<sub>2-6</sub> alkylcarbonyl or amino optionally substituted by C<sub>1-5</sub> alkyl;

10 R<sub>3</sub> is C<sub>1-5</sub> alkyl optionally substituted by the group -NHR<sub>7</sub>, wherein R<sub>7</sub> is hydrogen, C<sub>1-5</sub> alkyl or C<sub>2-6</sub> alkylcarbonyl; and

R<sub>4</sub> is phenyl optionally substituted by halogen, C<sub>1-5</sub> alkoxy, trifluoromethyl or C<sub>1-5</sub> alkyl.

15 The ACE inhibitor compounds of the present invention may also include phosphory aminoacid derivators as disclosed in EP Patent No. 0009183 (the entire contents of which are incorporated herein by reference), and having the general formula (XVII) as shown below:



20 wherein n is 0 or 1;

R is hydrogen, lower alkyl, phenyl lower alkyl, hydroxy phenyl lower alkyl, hydroxy lower alkyl, amino lower alkyl, guanidino lower alkyl, imidazoyl lower alkyl, indolyl lower alkyl, mercapto lower alkyl, lower alkyl mercapto lower alkyl;

R<sub>3</sub> is hydrogen;

5 R<sub>4</sub> is hydrogen, lower alkyl, phenyl lower alkyl, hydroxyl phenyl lower alkyl, hydroxyl lower alkyl, aminolower alkyl, guanidino lower alkyl, guanidino lower alkyl, imidazoyl lower alkyl, indolyl lower alkyl, mercapto lower alkyl, lower alkyl mercapto lower alkyl;

10 R<sub>3</sub> and R<sub>4</sub> may be connected together to form an alkylene bridge of from 2 to 4 carbon atoms or an alkylene bridge of from 2 to 3 carbon atoms one sulfur atom;

X is O, NR<sub>5</sub>, S where R<sub>5</sub> = H or lower alkyl;

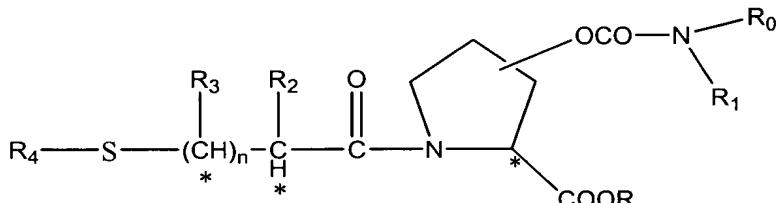
R<sub>1</sub> is hydrogen, lower alkyl, aralkyl or aryl;

R<sub>2</sub> is hydrogen, lower alkyl, aralkyl or aryl and pharmaceutically acceptable salts thereof.

15

The ACE inhibitors may also include the carbamate derivatives of mercaptoacyl hydroxyl prolines as disclosed in U.S. Patent No. 4,217,359 (the entire contents of which are incorporated herein by reference), and which have the formula (XVIII) as shown below:

20

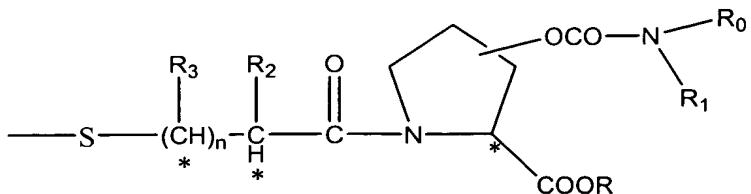


wherein R, R<sub>2</sub> an R<sub>3</sub> each is hydrogen or lower alkyl;

R<sub>0</sub> and R<sub>1</sub> each is hydrogen, lower alkyl, cyclo-lower alkyl, allyl, propargyl, phenyl or substituted phenyl; or R<sub>0</sub> and R<sub>1</sub> can join with the nitrogen to form a 5- or 6-membered heterocyclic;

25

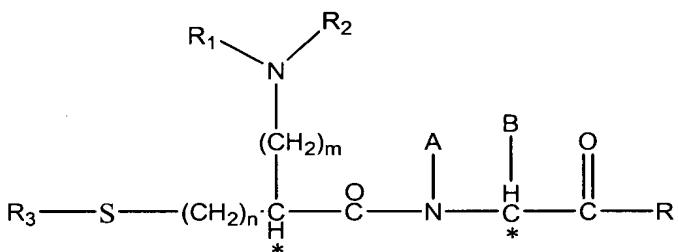
R<sub>4</sub> is hydrogen or hydrolysable organic protecting group of the formula R<sub>5</sub>-CO- or



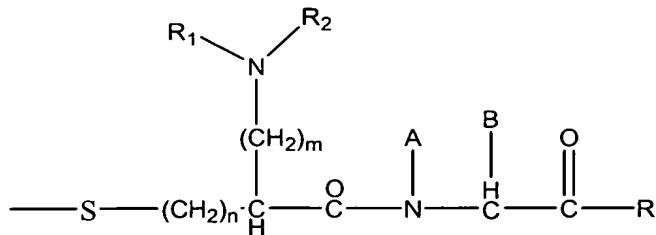
R<sub>5</sub> is lower alkyl, phenyl, substituted phenyl, phenyl-lower alkyl, substituted phenyl-lower alkyl, cycloalkyl, thienyl, or furyl;  
 5 n is 0, 1 or 2; and salts thereof.

The asterisks indicate centers of asymmetry. The carbon in the acyclic side chain is asymmetric when R<sub>2</sub> and/or R<sub>3</sub> is other than hydrogen. Each of the centers of asymmetry provide D and L forms which can be separated by conventional  
 10 methods as described below.

The ACE inhibitors of the present invention also may include substituted acyl derivatives of amino acids disclosed in U.S. Patent No. 4,129,571 (the entire contents of which are incorporated herein by reference), and which have the general  
 15 formula (XIX) as shown below:



and salts thereof, wherein:  
 R is hydroxyl or lower alkoxy;  
 R<sub>1</sub> is hydrogen, lower alkanoyl or amino(imino)-methyl;  
 20 R<sub>2</sub> is hydrogen, lower alkyl or phenyl-lower alkylene;  
 R<sub>3</sub> is hydrogen, lower alkanoyl, benzoyl or



A is hydrogen, lower alkyl or hydroxyl-lower alkylene;

B is hydrogen, lower alkyl, phenyl, phenyl-lower alkylene, hydroxyl-lower  
5 alkylene, hydroxyphenyl-lower alkylene, amino-lower alkylene, guanidino-lower  
alkylene, mercapto-lower alkylene, lower alkyl-thio-lower alkylene, imidazolyl-  
lower alkylene, indolyl-lower alkylene, carbamoyl-lower alkylene or carboxy-lower  
alkylene;

10 or A and B together form a  $(\text{CH}_2)_p$  bridge which completes a ring of 5 or 6 atoms with the nitrogen and carbon to which they are joined, one carbon optionally bearing a hydroxy group;

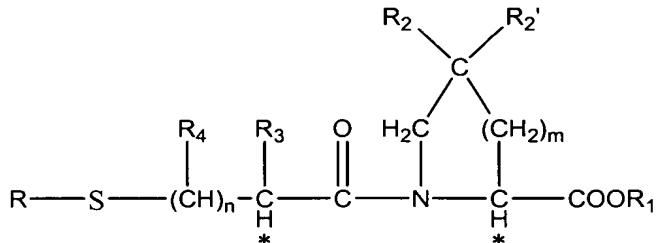
n is 0 or 1;

m 0, 1, 2, 3 or 4; at least one of m and n is other than 0; and

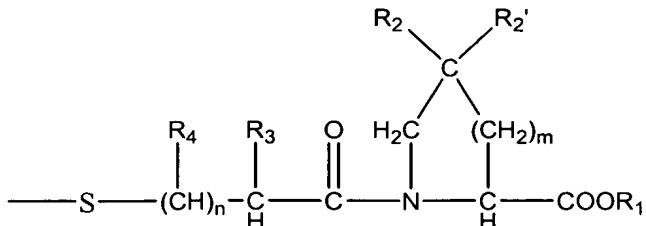
p is 3 or 4.

15 The asterisks denote centers of asymmetry.

ACE inhibitors may also include halogenated compounds as disclosed in U.S. Patent No. 4,154,935 (the entire contents of which are incorporated herein by reference), which have the general formula (XX) as shown below:



20 wherein R is hydrogen, lower alkanoyl or



R<sub>1</sub> is hydrogen or lower alkyl;

R<sub>2</sub> and R<sub>2'</sub> each independently represent hydrogen or halogen;

R<sub>3</sub> and R<sub>4</sub> each independently represent hydrogen, lower alkyl or

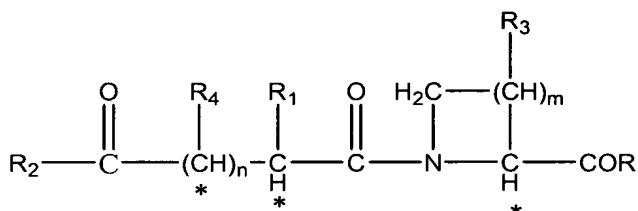
- 5 trifluoromethyl, not more than one being trifluoromethyl, and at least one of R<sub>2</sub>, R<sub>2'</sub>, R<sub>3</sub> or R<sub>4</sub> is a halogen or trifluoromethyl substituent represented by the named symbol as defined above;

m is 2; and

n is 0 or 1.

- 10 The asterisks indicate asymmetric carbon atoms.

ACE inhibitor compounds may also include carboxyalkylacylamino acids and related compounds disclosed in U.S. independently represent 4,052,511 (the entire contents of which are incorporated herein by reference), which are derivatives 15 of proline, pipecolic acid, azetidine-2-carboxylic acid and which have the general formula (XXI) as shown below:



wherein

R is hydroxy, amino or lower alkoxy;

- 20 R<sub>1</sub> and R<sub>4</sub> each is hydrogen, lower alkyl or phenyl-lower alkyl;

R<sub>2</sub> is hydroxy, amino, hydroxyamino or lower alkoxy;

R<sub>3</sub> is hydrogen, hydroxy or lower alkyl;

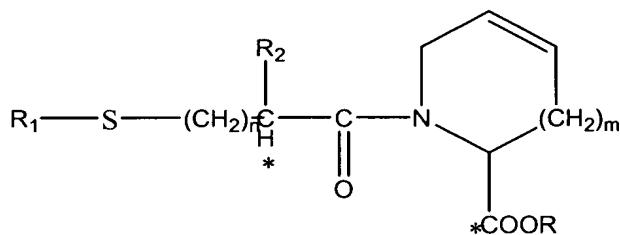
m is 1 to 3;

n is 0 to 2.

The asterisks indicate asymmetric carbon atoms. The carbons in the acyclic  
5 side chain are asymmetric when R<sub>1</sub> or R<sub>4</sub> are other than hydrogen.

The ACE inhibitor compounds may also include the dehydrocyclicimino acid  
compounds disclosed in U.S. independently represent 4,129,566 (the entire contents  
of which are incorporated herein by reference), which have the general formula

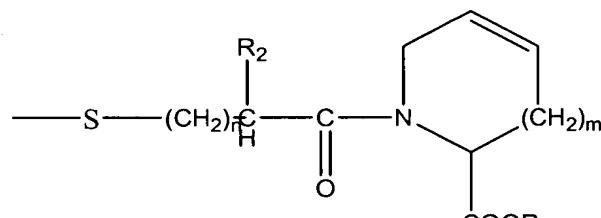
10 (XXII) as shown below:



wherein:

R and R<sub>2</sub> each is hydrogen or lower alkyl;

R<sub>1</sub> is hydrogen, lower alkanoyl or

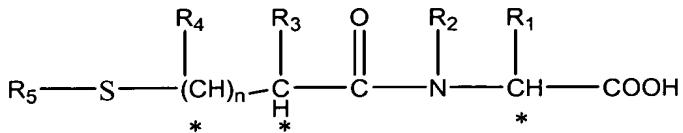


15

m and n each is 0 or 1.

The asterisks indicate asymmetric carbon atoms. The carbon in the acyclic  
side chain is asymmetric when R<sub>1</sub> is other than hydrogen.

The ACE inhibitor compounds may also include compounds disclosed in U.S. independently represent 4,053,651 (the entire contents of which are incorporated herein by reference), having the formula (XXIII) as shown below:

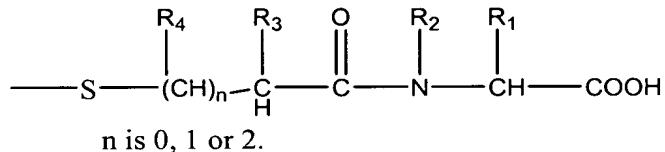


5 or a salt thereof, wherein

R<sub>1</sub> is hydrogen, lower alkyl, phenyl-lower alkylene, hydroxyl-lower alkylene, amino-lower alkylene, guanidino-lower alkylene, imidazolyl-lower alkylene, indolyl-lower alkylene, mercapto-lower alkylene, lower alkylmercapto-lower alkylene, carbamoyl-lower alkylene or carboxy-lower alkylene;

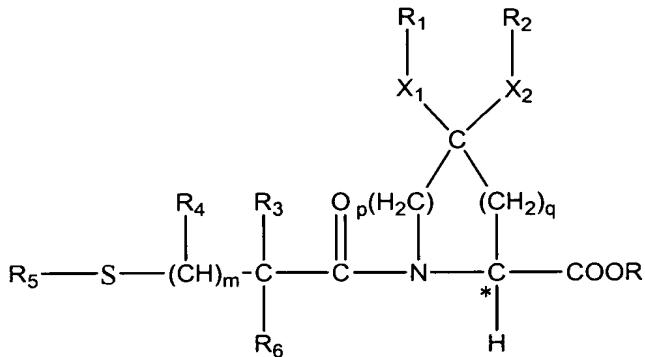
10 R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> each is hydrogen, lower alkyl or phenyl-lower alkylene;

R<sub>5</sub> is hydrogen, lower alkanoyl, benzoyl or



15 The asterisks denote centers of asymmetry.

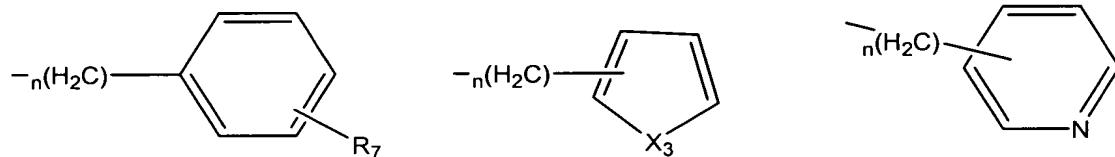
ACE inhibitors may also include derivatives of mercaptoacyl prolines and pipecolic acids as disclosed in U.S. independently represent 4,311,697 (the entire contents of which are incorporated herein by reference), of the formula (XXIV) as 20 shown below:



R and R<sub>6</sub> are independently selected from hydrogen and lower alkyl provided that R<sub>6</sub> is lower alkyl only if R<sub>3</sub> is also lower alkyl;

5      R<sub>3</sub> and R<sub>4</sub> are independently selected from hydrogen, lower alkyl, lower alkylthio, -(CH<sub>2</sub>)<sub>n</sub>-SH, and halo substituted lower alkyl;

X<sub>1</sub>, X<sub>2</sub> and X<sub>3</sub> are independently selected from lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, halo substituted lower alkyl, hydroxyl substituted lower alkyl;

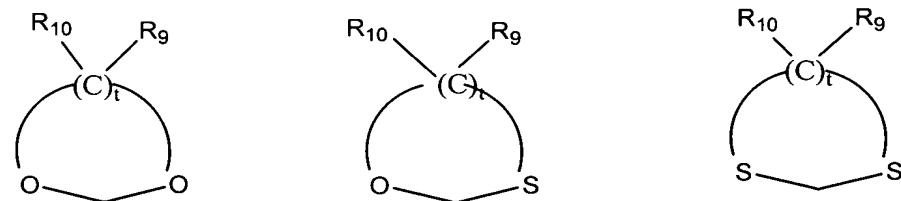


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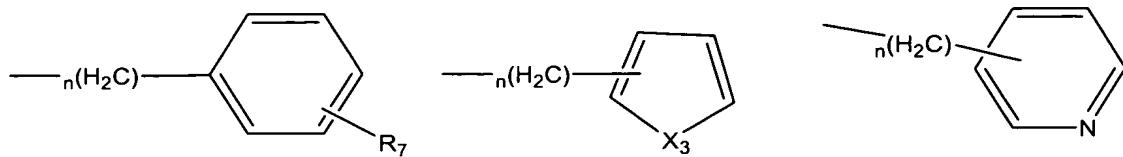
or R<sub>1</sub> and R<sub>2</sub> join in a polymethylene chain to complete an unsubstituted or substituted 5- or 6-membered ring.

When R<sub>1</sub> and R<sub>2</sub> are joined together in a polymethylene chain of 2 or 3 carbons, these cyclic ketal and thioketals can be represented as follows:

15



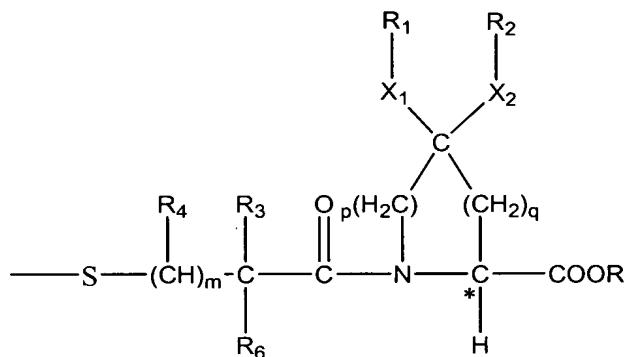
wherein t is 2 or 3 and R<sub>9</sub> and R<sub>10</sub> are both hydrogen, both lower alkyl, or one is hydrogen and the other is lower alkyl, halo substituted lower alkyl, hydroxyl substituted lower alkyl,



preferably, only one carbon of the polymethylene chain will be substituted.

- R<sub>7</sub> is hydrogen, lower alkyl of 1 to 4 carbons, especially methyl, lower  
 5 alkoxy of 1 to 4 carbons, especially methyl, lower alkoxy of 1 to 4 carbons,  
 especially methoxy, lower alkythio 1 to 4 carbons, especially methylthio, chloro,  
 bromo, fluoro, trifluoromethyl, or hydroxy;

- R<sub>5</sub> is hydrogen, a hydrolyzably removable protecting group, a chemically  
 removable protecting group, or when R<sub>3</sub> and R<sub>4</sub> are other than -(CH<sub>2</sub>)<sub>m</sub>-SH a  
 10 sulfide of the formula



m is zero, one or two;

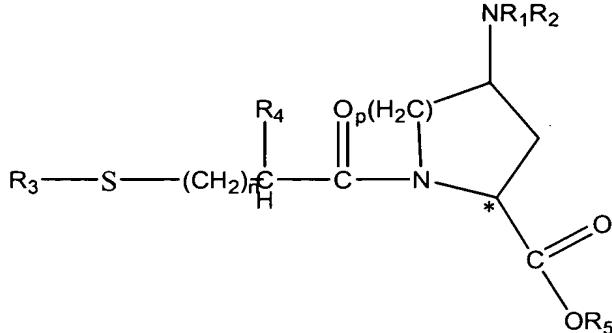
n is one, two or three;

p and q are each one or two provided that both are not two.

- 15 The asterisk in the above formula indicates a center of asymmetry in the ring.  
 In the case of praline, i.e., p and q are both one, this center is in the L-configuration.  
 In the case of pipecolic acid, i.e., one of p and q is two, this center is in the D, L or  
 L-configuration.

- Asymmetric centers can also be present in the mercaptoacyl sidechain  
 20 depending upon the definition of R<sub>3</sub>, R<sub>4</sub> and R<sub>6</sub>. Another asymmetric center may  
 also be present in the ring when X<sub>1</sub>-R<sub>1</sub> and X<sub>2</sub>-R<sub>2</sub> are different. The products can  
 accordingly exist in stereoisomeric forms or as racemic mixtures thereof.

The ACE inhibitors may also include the imido, amido and amino derivative compounds of mercaptoacyl prolines and pipecolic acids disclosed in U.S. Patent No. 4,310,461 (the entire contents of which are incorporated herein by reference),  
 5 and having the formula (XXV) as shown below:



and salts thereof, and the symmetrical dimer thereof, wherein

R<sub>1</sub> and R<sub>2</sub> are the same or different and are hydrogen, alkyl, cycloalkyl, 1-adamantyl, aryl, arylalkyl, alkylcarbonyl, arylcarbonyl, arylalkylcarbonyl,  
 10 alkysulfonyl, arysulfonyl, or arylvinylcarbonyl (aryl-CH=CH—CO--), or together with the nitrogen atom to which they are attached R<sub>1</sub> and R<sub>2</sub> are 1-pyrrolidinyl, 1-piperidinyl, 1-homopiperidinyl, 4-morpholinyl, 4-alkyl-1-piperazinyl, 4-aryl-1-piperazinyl, 1-imidazolyl, 1-pyrrolidinyl-2,5-dione(succinimido), 3-alkyl-1-pyrrolidinyl-2,5-dione, 3-aryl-1-pyrrolidinyl-2,5-dione, 1-piperidinyl-2,6-dione, 2H-15 isoindol-2-yl-1,3-dione(pthalimido), hexahydro-2H-isoindol-2-yl-1,3-dione(hexahydrophthalimido), 2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl(maleimido), 1,1,3-trioxo-1,2-benziso-thiazol-2(3H)-yl(2-saccharinyl), or 1,3-dihydro-1,3-dioxo-2H-benz[de]isoquinolin-2-yl(1,8-naphthalenedicarboximdo);

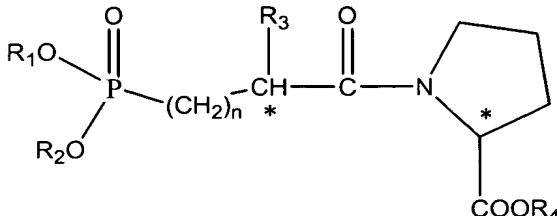
R<sub>3</sub> is hydrogen, alkyl, aryl, arylalkyl, or a hydrolyzable acyl protecting group  
 20 such as alkanoyl or arylcarbonyl;

R<sub>4</sub> is hydrogen, alkyl, alkylthio or trifluoromethyl;

R<sub>5</sub> is hydrogen, alkyl, or arylalkyl;

n is 0, 1 or 2; and p is 1 or 2.

The ACE inhibitors may also include phosphonoacyl prolines and related compounds as disclosed in U.S. Patent No. 4,151,172 (the entire contents of which are incorporated herein by reference), which have the formula (XXVI) as shown below:



5

R<sub>1</sub> and R<sub>2</sub> each is hydrogen, lower alkyl, unsubstituted or substituted phenyl-lower alkyl or a metal ion;

R<sub>3</sub> is hydrogen or lower alkyl;

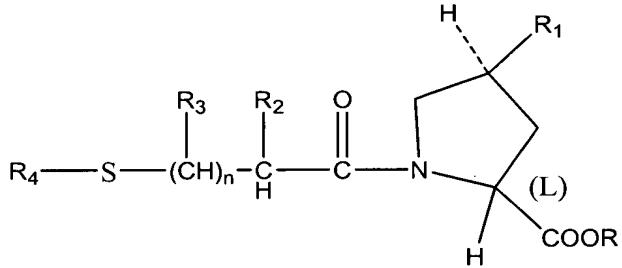
R<sub>4</sub> is hydrogen, lower alkyl, phenyl-lower alkyl or a metal ion; and

10

n is 0 or 1.

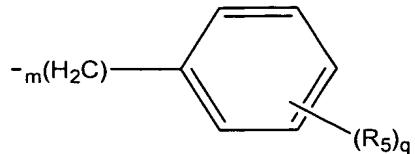
The ACE inhibitor compounds may also include mercaptoacyl derivatives of various 4-cis substituted prolines and salts thereof as disclosed in U.S. Patent No. 4,316,905 (the entire contents of which are incorporated herein by reference), and

15 which have the formula (XXVII) as shown below:

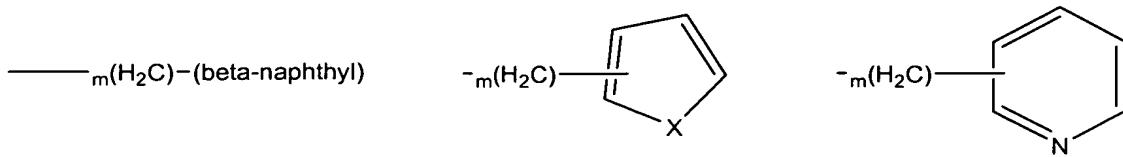


R represents hydrogen or lower alkyl;

R<sub>1</sub> represents -(CH<sub>2</sub>)<sub>m</sub>-cycloalkyl, 1-cyclohexenyl, 1,4-cyclohexadienyl;



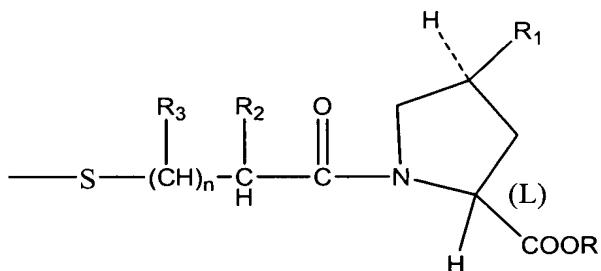
—<sub>m</sub>(H<sub>2</sub>C)—(alpha-naphthyl)



$R_2$  and  $R_3$  are independently selected from hydrogen, lower alkyl, lower alkylthio and halo substituted lower alkyl;

5         $n$  is zero, one or two;

$R_4$  is hydrogen, a hydrolyzably removable protecting group, a chemically removable protecting group, or



$m$  is zero, one, two or three;

10       $R_5$  is hydrogen, lower alkyl of 1 to 4 carbons, especially methyl, lower alkoxy of 1 to 4 carbons, especially methoxy, lower alkylthio of 1 to 4 carbons, especially methylthio, chloro, bromo, fluoro, trifluoromethyl, hydroxy, phenyl, phenoxy, phenylthio, or phenylmethyl. The hydroxy substituted compounds are obtained by heating the corresponding methoxy substituted compound with pyridine  
15       $HC1$ ;

$q$  is one, two or three provided that  $q$  is more than one only if  $R_5$  is hydrogen, methyl, methoxy, chloro or fluoro;

$X$  is oxygen or sulfur.

20      The ACE inhibitors also preferably include: aylmercapto and mercaptoalkanoyl prolines (U.S. Patent No. 4,046,889) such as captopril (1-[(2S)-3-mercaptop-2-methylpropionyl]-L-proline) (U.S. Patent No. 4,105,776) and ether or thioether mercaptoacyl prolines such as zofenopril (U.S. Patent No. 4,316,906);

carboxyalkyl dipeptides such as enalapril (N-(1-ethoxycarbonyl-3-phenylpropyl)-L-ananyl-L-proline) (U.S. Patent No. 4,374,829), lisinopril (U.S. Patent No. 4,374,829), quinapril (U.S. Patent No. 4,344,949), and ramipril (U.S. Patent No. 4,508,729); carboxyalkyl dipeptide mimics such as cilazapril (U.S. Patent No. 5 4,512,924) and benazapril (U.S. Patent No. 4,410,520); phosphinylalkanoyl prolines (U.S. Patent No. 4,168,267) such as fosinopril (U.S. Patent No. 4,337,201) and trandolopril; phosphonamide substituted amino or imino acids (U.S. Patent No. 4,432,971); phosphonate substituted amino or imino acids and salts thereof, including ceronapril ((S)-1-[6-amino-2-[[hydroxyl(4-phenylbutyl)phosphinyl]oxy]-10 1-oxohexyl]-L-proline) (U.S. Patent No. 4,452,790); Beecham's BRL 36,378 (EP Nos. 80822 and 60668); Chugai's MC-838 (disclosed in CA 102:72588v and Jap.J.Pharmacol. 40:373 (1986)); Ciba-Geigy's CGS 14824 (3-([1-ethoxycarbonyl-15 3-phenyl-(1S)-propyl]-amino)-2,3,4,5-tetrahydro-2-oxo-1-(3S)-benzazepine-1 acetic acid HCL) (U.K. Patent No. 2103614) and CGS 16,617 (3(S)-[[1S)-5-amino-1-carboxypentyl]amino]2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-1-ethanoic acid) (U.S. Patent No. 4,473,575); Cetapril (alacepril, Dainippon) (Eur.Therap.Res. 39:671 (1986); 40:543 (1986)); Ru 44570 (Hoechst) (Arzneimittelforschung 35:1254 (1985)); Cilazapril (Hoffman-LaRoche) (J.Cardiovasc.Pharmacol. 9:39 (1987); Ro 31-2201 (Hoffman-LaRoche) (FEBS Lett. 165:201 (1984); Lisinopril 20 20 (Merck) (Curr.Therap.Res. 37:342 (1985) and Eur. Patent App. No. 12-401); Indalapril (delapril) (U.S. Patent No. 4,385,051); Rentiapril (fentiapril, Santen) (Clin.Exp. Pharmacol. Physiol. 10:131 (1983); Indolapril (Schering) (J.Cardiovasc.Pharmacol. 5:643, 655 (1983)); Spirapril (Schering) (Acta.Pharmacol.Toxicol. 59 (Supp. 5):173 (1986); Perindopril (Servier) 25 (Eur.J.Clin.Pharmacol. 31:519 (1987); Quinapril (Warner-Lambert) (U.S. Patent No. 4,344,949); CI 925 (Warner-Lambert) ([3S-[2[R(\*)R(\*)]]]3R(\*)]-2-[2-[[1-(ethoxy-carbonyl)-3-phenylpropyl]amino[-1-oxopropyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid HCL) (Pharmacologist 26:243, 266 (1984)); WY-44221 (Wyeth) (J.Med.Chem. 26:394 (1983)); Mercapto containing compounds such as 30 pivopril and YS980 (U.S. Patent No. 6,127,370); Omapatrilat (Drugs R.D. 1999 1(4):350-1) (U.S. Patent No. 6,300,503); Alacepril (Jap. Patent App. No. 78/82809); moveltopril; quinaprilat; moexipril; perinodpril (S-9490) (Annual Drug Data Report

7, 99 (1985)); pentopril; ancovenin (Annual Drug Data Report 6, 20 (1984)); phenacein (Annual Drug Data Report 7, 20 (1985)); and nicotianamin (East German Patent DD 226 880). The contents of all cited patents, applications, publications are hereby incorporated herein by reference.

5       The most preferred ACEI is Enalapril, i.e. (N-(1-ethoxycarbonyl-3-phenylpropyl)-L-ananyl-L-proline), (U.S. Patent No. 4,374,829, entire contents incorporated herein by reference) and other similar or derivative carboxyalkyl dipeptides. Other preferred ACE inhibitors include lisinopril or captopril.

(iii) Angiotensin II Receptor Blockers / Antagonists

10       Angiotensin is formed from a precursor, angiotensinogen, which is produced by the liver and found in the alpha-globulin fraction of plasma. The lowering of blood pressure is a stimulus to secretion of renin by the kidney into the blood. Renin cleaves from angiotensinogen a terminal decapeptide, angiotensin I. This is further altered by the enzymatic removal of a dipeptide, by Angiotensin Convertin Enzyme 15 (ACE), to form angiotensin II. Angiotensin II is a potent regulator of blood pressure and of water and electrolyte balance. Angiotensin II interacts with two pharmacologically distinct subtypes of cell surface receptors, types 1 and 2. Whereas AGTR1, the type-1 receptor for Angiotensin II, mediates the vasopressive and aldosterone-secreting effects of angiotensin II, the function of the type-2 20 Angiotensin receptor (AGTR2) was relatively unclear, although it is expressed in both adult and embryonic life. Recent evidence indicates that the type-2 Angiotension receptor is not required for embryonic development, but plays a role in the central nervous system and cardiovascular functions that are mediated by the renin-angiotensin system (Hein et al., *Nature* 377: 744-748, 1995).

25       Ichiki et al. (*Nature* 377: 748-750, 1995) reported the unexpected finding that the targeted disruption of the mouse AGTR2 gene resulted in a significant increase in blood pressure and increased sensitivity to the pressor action of angiotensin II. The authors concluded that the type 2 receptor mediates a depressor effect and antagonizes the AGTR1-mediated pressor action of angiotensin II. In 30 addition, disruption of the AGTR2 gene attenuated exploratory behavior and lowered blood pressure. Their results indicated that angiotensin II activates AGTR1

and AGTR2, which have mutually counteracting hemodynamic effects, and that AGTR2 regulates central nervous system functions, including behavior. Ichiki et al. (1995) commented on the fact that Hein et al. (1995) did not find an increase in basal blood pressure and they suggested that this could be due to differences in 5 genetic background of the mice studied.

Angiotensin-II receptor antagonists act by binding to specific membrane-bound receptors that displace Angiotensin II from its type 1-receptor subtype (AGTR1). These drugs therefore function as selective blockers. AT-II pressor effects are mediated by AGTR1. Unlike angiotensin-converting enzyme inhibitors, 10 they do not inhibit bradykinin metabolism or enhance prostaglandin synthesis. Angiotensin-II receptor antagonists are well tolerated. Cough occurs much less often with these agents than with angiotensin-converting enzyme inhibitors, and they do not adversely affect lipid profiles or cause rebound hypertension after discontinuation. There has been a rapid growth in members of this new class of 15 drugs. The angiotensin-II receptor antagonists that have been labeled for use in hypertension by the U.S. Food and Drug Administration (FDA) are Losartan (Cozaar®), Valsartan (Diovan®), Irbesartan (Avapro®), Candesartan (Atacand®) and Telmisartan (Micardis®). Other angiotensin-II receptor antagonists currently under investigation include tasosartan, zolarsartan, Teveten (eprosartan mesylate). At the 20 present time four are being actively marketed in Canada: Losartan (Cozaar®), Valsartan (Diovan®), Irbesartan (Avapro®), Candesartan (Atacand®).

**Losartan.** Losartan (U.S. Pat. No. 5,153,197) was the first angiotensin-II receptor antagonist to be introduced (1995). Compared with the parent drug, the active metabolite (EXP3174) has a longer half-life and antihypertensive effects that 25 correlate more with plasma concentration. Double-blind studies have shown that losartan is well tolerated and as efficacious as enalapril and nifedipine for lowering blood pressure. The mean blood pressure reduction achieved with losartan in a dosage of 50 to 150 mg once daily is 5.5 to 10.5 mm Hg for systolic pressure and 3.5 to 7.5 mm Hg for diastolic pressure. One review of the efficacy and safety of 30 losartan in the treatment of essential hypertension indicated a slowly developing

response, with blood pressure becoming lower over several weeks of continued treatment.

The starting dosage of losartan is 50 mg once daily. The duration of activity for a dose is 24 hours. Twice-daily dosing can be used if the antihypertensive effect measured at a trough is inadequate. However, a comparison of losartan in dosages of 5 100 mg once daily and 50 mg twice daily showed no significant difference in antihypertensive efficacy.

A hydrochlorothiazide-losartan combination (Hyzaar) is also available. This combination drug contains 12.5 mg of hydrochlorothiazide and 50 mg of losartan.

10 Some investigators advocate the use of this combination instead of escalation of a single drug, because dose-dependent adverse effects are less likely to occur. Dosing is once or twice daily.

**Valsartan.** (U.S. Pat. Nos. 5,399,578; 6,294,197) Placebo-controlled trials have found valsartan to be both safe and effective for the treatment of hypertension.

15 With valsartan taken in a dosage of 80 to 320 mg once daily, the mean reduction in diastolic blood pressure is 6 to 9 mm Hg, and the mean reduction in systolic pressure is 3 to 6 mm Hg. Studies have shown that valsartan is as effective as ACE inhibitors enalapril, lisinopril and amlodipine in the treatment of mild to moderate hypertension.

20 The affinity of valsartan for the AT<sub>1</sub> receptor is about 20,000 times greater than its affinity for the AT<sub>2</sub> receptor. In comparison, the affinity of losartan for the AT<sub>1</sub> receptor is about 1,000 times greater than its affinity for AT<sub>2</sub> receptors. The clinical implication of receptor affinity is not yet clear.

25 Valsartan is also available as a combination product with hydrochlorothiazide (Diovan HCT). This combination drug contains 80 or 160 mg of valsartan and 12.5 mg of hydrochlorothiazide. With the addition of hydrochlorothiazide, blood pressure decreases even more (i.e., by 6 mm Hg systolic and 3 mm Hg diastolic). Dosing is once daily.

**Irbesartan.** Irbesartan (U.S. Pat. Nos. 5,270,317; 5,994,348; 6,342,247) is a 30 safe and effective angiotensin-II receptor antagonist with an affinity for the AT<sub>1</sub>

receptor that is more than 8,500 times greater than its affinity for the AT<sub>2</sub> receptor. This agent has a higher bioavailability (60 to 80 percent) than other drugs in its class.

In one study, 530 patients with mild to moderate hypertension were given  
5 placebo, losartan in a dosage of 100 mg per day or irbesartan in a dosage of 150 or 300 mg per day. After only one week of therapy, blood pressure trough reduction was significantly greater with irbesartan in a dosage of 300 mg per day than with losartan in a dosage of 100 mg per day.

Placebo-controlled trials have shown that irbesartan in a dosage of 150 to  
10 300 mg per day lowers mean systolic blood pressure by 8 to 12 mm Hg and mean diastolic pressure by 5 to 8 mm Hg. Irbesartan has also been found to be as effective as enalapril and atenolol in reducing blood pressure.

A combination product that contains both irbesartan and hydrochlorothiazide is being developed.

15        **Candesartan.** Candesartan cilexetil (U.S. Pat. No. 5,196,444) has been shown to be effective for the treatment of hypertension. Candesartan itself is poorly absorbed after oral administration; the ester prodrug, candesartan cilexetil, improves bioavailability. With oral administration of candesartan cilexetil, conversion to the active compound occurs rapidly and completely during gastrointestinal absorption.  
20        The affinity of candesartan for the AT<sub>1</sub> receptor is more than 10,000 times greater than its affinity for the AT<sub>2</sub> receptor.

Candesartan is both safe and well tolerated in dosages of 8 to 32 mg per day. With these dosages, systolic blood pressure is reduced by 8 to 12 mm Hg and diastolic pressure is reduced by 4 to 8 mm Hg.

25        Comparable reductions of diastolic blood pressure have been achieved with candesartan in a dosage of 8 mg per day and enalapril in a dosage of 10 mg per day. In one trial, significant reductions in mean sitting diastolic pressures occurred after 12 weeks of treatment with candesartan in a dosage of 8 or 12 mg per day and enalapril in a dosage of 10 mg per day ( $P < 0.01$ ), but not with candesartan in a  
30 dosage of 4 mg per day ( $P = 0.074$ ). The same study compared losartan in a dosage

of 50 mg per day with candesartan in dosages of 8 and 16 mg per day. The 16-mg dosage of candesartan reduced diastolic blood pressure by an adjusted mean of 3.7 mm Hg more than the 50-mg losartan dosage.

**Telmisartan.** Telmisartan (U.S. Pat. No. 5,591,762) is the most recently 5 labeled angiotensin-II receptor antagonist. Its affinity for the AT<sub>1</sub> receptor is more than 3,000 times greater than its affinity for the AT<sub>2</sub> receptor. Nonlinear pharmacokinetics yield a greater than proportional increase in plasma telmisartan concentrations with increasing dosages.

The efficacy of telmisartan in the treatment of hypertension has been 10 demonstrated in placebo-controlled trials. A three-month study of 440 patients showed that telmisartan in a dosage of 40, 80, 120 or 160 mg per day produced a slightly greater antihypertensive effect than enalapril in a dosage of 20 mg per day. In this study, diastolic blood pressure reductions with telmisartan ranged from 8.6 to 9.3 mm Hg, and systolic blood pressure reductions ranged from 10 to 11.9 mm Hg. 15 The decreases in diastolic and systolic blood pressures for enalapril were 7.2 and 8.2 mm Hg, respectively.

Like the other angiotensin-II receptor antagonists, telmisartan has been shown to have a side effect profile similar to that of placebo. Clinical trials have demonstrated no rebound hypertension or first-dose orthostatic effect.

20 Most recently, the FDA has approved a new angiotensin II receptor blocker called olmesartan medoxomil (Benicar), for the treatment of hypertension. A 20 mg-starting dose of olmesartan medoxomil has been shown to reduce systolic pressure by an average of 15 mm Hg and diastolic pressure by an average of 12 mm Hg. The manufacturers Sankyo Pharma Inc. stated that studies have shown their drug to be 25 superior to losartan, and the launch of Benicar is expected within the first half of 2002.

In addition, Iyer et al. (*Proc. Nat. Acad. Sci.* 93: 9960-9965, 1996) explored 30 the possibility of using gene therapy to inhibit AGTR1. They demonstrated that the delivery of angiotensin type 1 receptor antisense by a retrovirally-mediated delivery system resulted in a selective attenuation of the cellular actions of angiotensin II in the neurons of the spontaneously hypertensive (SH) rat model. A single injection

normalized blood pressure in the SH rat on a long-term basis. The use of this approach in patients was proposed. Thus, this type of AGTR1 antagonist is also within the scope of the AIIRA of the instant invention.

Although both ACE inhibitors and AIIRAs prevent the activation of AGTR1,  
5 there is a difference between the effects of these two classes of compounds. This is because non-ACE pathways can also produce some angiotensin II. ACE inhibitors also decrease bradykinin breakdown and this action could be involved in some of the beneficial and adverse effects of that class of drugs. Therefore, a potential for differential clinical effects exists for these two classes of drugs. For example,  
10 angiotensin receptor blockers are indicated in patients who require an ACE inhibitor but who cannot tolerate it due to drug-induced dry cough. Similar consideration can also be helpful in conjoint administration of morphogen with either ACE inhibitor or AIIRA.

*B. Formulations and Methods of Treatment*

15 In one embodiment, the invention comprises a pharmaceutical composition comprising a therapeutically effective amount an ACE inhibitor and an OP/BMP morphogen formulated with pharmaceutically acceptable salt, carrier, excipient or diluent. In one embodiment, the ACE inhibitor is Enalapril. In another embodiment, the ACE ACEI is: any one compound of the formulas I-XXVIII or  
20 their salts thereof; acylmercapto and mercaptoalkanoyl prolines; captopril (1-[(2S)-3-mercaptopropanoyl]-L-proline); ether or thioether mercaptoacyl prolines; zofenopril; carboxyalkyl dipeptides; enalapril (N-(1-ethoxycarbonyl-3-phenylpropyl)-L-ananyl-L-proline); lisinopril; quinapril; ramipril; carboxyalkyl dipeptide mimics; cilazapril; benazapril; phosphinylalkanoyl prolines; fosinopril;  
25 trandolopril; phosphonamide substituted amino or imino acids; phosphonate substituted amino or imino acids and salts thereof; ceronapril ((S)-1-[6-amino-2-[[hydroxyl(4-phenylbutyl)phosphinyl]oxy]-1-oxohexyl]-L-proline); BRL 36,378; MC-838; CGS 14824 (3-((1-ethoxycarbonyl-3-phenyl-(1S)-propyl)-amino)-2,3,4,5-tetrahydro-2-oxo-1-(3S)-benzazepine-1 acetic acid HCL); CGS 16,617 (3(S)-[(1S)-  
30 5-amino-1-carboxypentyl]amino]2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-1-ethanoic acid); Cetapril (alacepril, Dainippon); Ru 44570; Cilazapril; Ro 31-2201;

Lisinopril; Indalapril (delapril); Rentiapril (fentiapril, Santen); Indolapril; Spirapril; Perindopril; Quinapril; CI 925 ([3S-[2[R(\*)R(\*)]]3R(\*)]-2-[2-[[1-(ethoxy-carbonyl)-3-phenylpropyl]amino[-1-oxopropyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid HCL); WY-44221; mercapto-containing compounds; 5 pivopril; YS980; Omapatrilat; Alacepril; moveltopril; quinaprilat; moexipril; perinodpril (S-9490); pentopril; ancovenin; phenacein; or nicotianamin.

In another embodiment, the invention comprises a pharmaceutical composition comprising a therapeutically effective amount an AIIRA and an OP/BMP morphogen formulated with pharmaceutically acceptable salt, carrier, 10 excipient or diluent. In one embodiment AIIRA is: Losartan (Cozaar<sup>®</sup>), Valsartan (Diovan<sup>®</sup>), Irbesartan (Avapro<sup>®</sup>), Candesartan (Atacand<sup>®</sup>), Telmisartan (Micardis<sup>®</sup>), tasosartan, zolarsartan, Teveten (eprosartan mesylate) or olmesartan medoxomil (Benicar).

15 In one embodiment, the morphogen in any of the above pharmaceutical composition embodiments is the polypeptide of SEQ ID NO: 3.

In another embodiment, the morphogen in any of the above pharmaceutical composition embodiments is a first polypeptide including at least a C-terminal cysteine domain of a protein selected from: a pro form, a mature form, or a soluble 20 form of a second polypeptide, wherein said second polypeptide is: OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, or BMP9.

In another embodiment, the morphogen in any of the above pharmaceutical composition embodiments comprises a polypeptide having at least 70% homology 25 or 50% identity with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-1 (SEQ ID NO: 2). In another embodiment, the polypeptide has at least 75% homology or 60% identity with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-1 (SEQ ID NO: 2). In yet another embodiment, the polypeptide has at least 80% homology or 70% identity with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-1 (SEQ ID NO: 2). In yet another embodiment, the polypeptide has at least 90% identity

with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-1 (SEQ ID NO: 2).

The invention also comprises a package pharmaceutical comprising any of the pharmaceutical compositions described herein, in association with instructions  
5 for administering the composition to a mammal for treatment or prevention of chronic renal failure.

The invention also comprises a package pharmaceutical comprising any of the pharmaceutical compositions described herein, in association with instructions for administering the composition to a mammal for delaying the need or reducing  
10 the frequency of chronic dialysis treatments.

In one embodiment, the invention provides a method of treating or preventing chronic renal failure in a mammal, comprising conjointly administering to said mammal (i) an OP/BMP morphogen, an inducer of endogenous OP/BMP morphogen expression, or an agonist of an OP/BMP morphogen receptor; and (ii) an  
15 Angiotensin-Converting Enzyme Inhibitor (ACEI).

In one embodiment, the invention provides a method of treating or preventing chronic renal failure in a mammal, comprising conjointly administering to said mammal (i) an OP/BMP morphogen, an inducer of endogenous OP/BMP morphogen expression, or an agonist of an OP/BMP morphogen receptor; and (ii)  
20 an Angiotensin- II Receptor Antagonist (AIIRA).

In another embodiment, the invention provides a method of treating or preventing chronic renal failure in a mammal, comprising introducing into the kidney of said mammal a therapeutically effective amount of renal mesenchymal progenitor cells pre-treated conjointly with an ACEI and an agent that increases the  
25 abundance of an OP/BMP morphogen.

In another embodiment, the invention provides a method of treating or preventing chronic renal failure in a mammal, comprising introducing into the kidney of said mammal a therapeutically effective amount of renal mesenchymal progenitor cells pre-treated conjointly with an AIIRA and an agent that increases the

abundance of an OP/BMP morphogen. In one embodiment the agent is an OP/BMP morphogen. In another embodiment the agent is an inducer of an OP/BMP morphogen. In another embodiment the agent is an agonist of an OP/BMP morphogen receptor.

5       In another embodiment the invention provides for a method for delaying the need for, or reducing the frequency of, chronic dialysis treatments, comprising conjointly administering to a mammal: (i) an OP/BMP morphogen, an inducer of endogenous OP/BMP morphogen expression, or an agonist of an OP/BMP morphogen receptor; and (ii) an ACEI.

10       In another embodiment the invention provides for a method for delaying the need for, or reducing the frequency of, chronic dialysis treatments, comprising conjointly administering to a mammal: (i) an OP/BMP morphogen, an inducer of endogenous OP/BMP morphogen expression or an agonist of an OP/BMP morphogen receptor; and (ii) an AIIRA.

15       In any of the above mentioned methods of treatment or prevention, said mammal may be afflicted with a condition selected from: chronic renal failure (CRF), end-stage renal disease (ESRD), chronic diabetic nephropathy, diabetic glomerulopathy, diabetic renal hypertrophy, hypertensive nephrosclerosis, hypertensive glomerulosclerosis, chronic glomerulonephritis, hereditary nephritis, or  
20       renal dysplasia.

In any of the above mentioned methods of treatment or prevention, the examination of a renal biopsy of said mammal may indicate that said mammal is afflicted with a condition selected from: glomerular hypertrophy, tubular hypertrophy, glomerulosclerosis, or tubulo interstitial sclerosis.

25       In any of the above mentioned methods of treatment or prevention, the examination of a renal biopsy of said mammal may indicate renal fibrosis. In one embodiment the examination may be by ultrasound, NMR or CAT scan of said mammal.

The invention also comprises use of: (i) an OP/BMP morphogen, an inducer of endogenous OP/BMP morphogen expression, or an agonist of an OP/BMP morphogen receptor; and (ii) an Angiotensin-Converting Enzyme Inhibitor (ACEI) for the preparation of a medicament for treating or preventing chronic renal failure  
5 in a mammal.

The invention also comprises use of: (i) an OP/BMP morphogen, an inducer of endogenous OP/BMP morphogen expression, or an agonist of an OP/BMP morphogen receptor; and (ii) an Angiotensin- II Receptor Antagonist (AIIRA) for the preparation of a medicament for treating or preventing chronic renal failure in a  
10 mammal.

In another embodiment the invention provides for use of mesenchymal progenitor cells that have been pretreated with an ACEI and an agent that increases the abundance of an OP/BMP morphogen for the preparation of a medicament to be introduced into the kidney of a mammal for treating or preventing chronic renal  
15 failure in a mammal.

In another embodiment the invention provides for use of mesenchymal progenitor cells that have been pretreated with an AIIRA and an agent that increases the abundance of an OP/BMP morphogen for the preparation of a medicament to be introduced into the kidney of a mammal for treating or preventing chronic renal  
20 failure in a mammal. In one embodiment the agent is an OP/BMP morphogen. In another embodiment the agent is an inducer of an OP/BMP morphogen. In another embodiment the agent is an agonist of an OP/BMP morphogen receptor.

The invention also comprises use of an (i) OP/BMP morphogen, an inducer of endogenous OP/BMP morphogen expression, or an agonist of an OP/BMP morphogen receptor; and (ii) an ACEI to prepare a medicament for delaying or  
25 reducing the frequency of chronic dialysis treatments in a mammal.

The invention also comprises use of an (i) OP/BMP morphogen, an inducer of endogenous OP/BMP morphogen expression, or an agonist of an OP/BMP

morphogen receptor; and (ii) an AIIRA to prepare a medicament for delaying or reducing the frequency of chronic dialysis treatments in a mammal.

In any of the above mentioned embodiments, said mammal may possess a number of functional nephron units which is less than about 40% of a number of 5 functional nephron units present in a mammal having intact healthy kidneys. In one embodiment, said mammal possesses a number of functional nephron units which is less than about 20% of a number of functional nephron units present in a mammal having intact healthy kidneys.

10 In any of the above mentioned embodiments, said mammal may be a kidney transplant recipient. In one embodiment, said mammal possesses only one kidney.

In any of the above mentioned embodiments, examination of a urinary sediment of said mammal may indicate a presence of broad casts.

15 In some of the above mentioned embodiments, said mammal may have a GFR which is chronically less than about 40% of a  $GFR_{exp}$  for said mammal. In some of the above mentioned embodiments, said mammal may have a GFR which is chronically less than about 20% of a  $GFR_{exp}$  for said mammal.

20 In any of the above mentioned embodiments, said mammal may be a human male weighing at least about 50 kg and has a GFR which is chronically less than about 40 ml/min. In any of the above mentioned embodiments, said mammal may be a human female weighing at least about 40 kg and has a GFR which is chronically less than about 30 ml/min.

In any of the above mentioned embodiments, said method of treatment or prevention, or said medicament, may reduce serum creatinine levels in said mammal by at least about 5% over 3 months.

25 In any of the above mentioned embodiments, prior to said treatment or prevention, said mammal may present a chronic decline in a clinical indicator of renal function, and after at least about 3 months of said treatment or prevention, said indicator may stabilize.

In any of the above mentioned embodiments, at least one of said ACEI, said AIIRA or said morphogen may be administered orally, parenterally, intravenously, intraperitoneally, or into a renal capsule, or by an implanted device. In any of the above mentioned embodiments, a stent may be implanted into said mammal for said 5 administration of at least one of said ACEI, said AIIRA or said morphogen.

In any of the above mentioned embodiments, at least one of said ACEI or said AIIRA, and at least one of said morphogen may be conjointly administered at least once a week for a period of at least about one month.

10 In any of the above mentioned embodiments, at least one of said ACEI or AIIRA, and at least one of said morphogen may be conjointly administered at least once a week for a period of at least about one year.

In any of the above mentioned embodiments, said ACEI or said AIIRA, and said morphogen may be administered: (i) through different routes or (ii) at different frequencies.

15 In any of the above mentioned embodiments, said morphogen may be administered at a dosage of about 0.01-1000 µg/kg body weight of said mammal.

In any of the above mentioned embodiments, said morphogen may be administered at a dosage of about 10-300 µg/kg body weight of said mammal.

20 In any of the above mentioned embodiments that comprises the administration or use of ACEI, said ACEI may be administered orally at a concentration of about 1-10,000 mg/L, preferably 10-1000 mg/L, 10-100 mg/L, 100-1000 mg/L, most preferably 100 mg/L.

25 In any of the above mentioned embodiments that comprises the administration or use of AIIRA, said AIIRA may be administered orally at a concentration of about 0.01-100 mg/kg body weight, preferably 0.1-10 mg/kg body weight, 0.2-5 mg/kg body weight, 0.5-2 mg/kg body weight, most preferably 1 mg/kg body weight.

In any of the above mentioned embodiments, said OP/BMP morphogen and, ACEI or AIIRA may be administered in a single pharmaceutical composition. In any of the above mentioned embodiments, said OP/BMP morphogen and, ACEI or AIIRA may be administered in separate pharmaceutical compositions at or around 5 the same time. In any of the above mentioned embodiments, said OP/BMP morphogen and, ACEI or AIIRA may be administered in separate pharmaceutical compositions at different times.

In any of the above mentioned embodiments, said morphogen may: (a) induce chondrogenesis in an ectopic bone assay; (b) prevent, inhibit, delay or 10 alleviate loss of renal function in an animal model of chronic renal failure, or (c) cause a clinically significant improvement in a standard marker of renal function when administered to a mammal in, or at risk of, chronic renal failure.

In any of the above mentioned embodiments, said morphogen may comprise 15 a polypeptide including at least a C-terminal cysteine domain of a protein selected from: a pro form, a mature form, or a soluble form of a polypeptide, wherein said polypeptide is: OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, or BMP9.

In any of the above mentioned embodiments, said morphogen may comprise a polypeptide including at least a C-terminal cysteine domain of a polypeptide selected from: a pro form, a mature form, or a soluble form of human OP-1.

20 In one embodiment, the morphogen used in any of the above mentioned embodiments may comprise a polypeptide having at least 70% homology or 50% identity with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-1 (SEQ ID NO: 2). In another embodiment, the morphogen used in any of the above mentioned embodiments may comprise a polypeptide having at least 25 75% homology or 60% identity with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-1 (SEQ ID NO: 2). In another embodiment, the morphogen used in any of the above mentioned embodiments may comprise a polypeptide having at least 80% homology or 70% identity with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-1 (SEQ ID NO: 2).  
30 In another embodiment, the morphogen used in any of the above mentioned

embodiments may comprise a polypeptide having at least 90% identity with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-1 (SEQ ID NO: 2).

- In any of the above mentioned embodiments said ACEI may be: any one  
5 compound of the formulas I-XXVIII or their salts thereof; acylmercapto and mercaptoalkanoyl prolines; captopril (1-[(2S)-3-mercaptopro-2-methylpropionyl]-L-proline); ether or thioether mercaptoacyl prolines; zofenopril; carboxyalkyl dipeptides; enalapril (N-(1-ethoxycarbonyl-3-phenylpropyl)-L-ananyl-L-proline); lisinopril; quinapril; ramipril; carboxyalkyl dipeptide mimics; cilazapril; benazapril; 10 phosphinylalkanoyl prolines; fosinopril; trandolopril; phosphonamide substituted amino or imino acids; phosphonate substituted amino or imino acids and salts thereof; ceronapril ((S)-1-[6-amino-2-[[hydroxyl(4-phenylbutyl)phosphinyl]oxy]-1-oxohexyl]-L-proline); BRL 36,378; MC-838; CGS 14824 (3-([1-ethoxycarbonyl-3-phenyl-(1S)-propyl]-amino)-2,3,4,5-tetrahydro-2-oxo-1-(3S)-benzazepine-1-acetic acid HCL); CGS 16,617 (3(S)-[[1S)-5-amino-1-carboxypentyl]amino]2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-1-ethanoic acid); Cetapril (alacepril, Dainippon); Ru 44570; Cilazapril; Ro 31-2201; Lisinopril; Indalapril (delapril); Rentiapril (fentiapril, Santen); Indolapril; Spirapril; Perindopril; Quinapril; CI 925 ([3S-[2[R(\*)]R(\*)]]3R(\*)]-2-[2-[[1-(ethoxy-carbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid HCL);  
20 WY-44221; mercapto-containing compounds; pivopril; YS980; Omapatrilat; Alacepril; moveltropril; quinaprilat; moexipril; perinodopril (S-9490); pentopril; ancovenin; phenacein; or nicotianamin. In a preferred embodiment the ACEI is Enalapril.
- 25 In any of the above mentioned embodiments said AIIRA may be: Losartan (Cozaar®), Valsartan (Diovan®), Irbesartan (Avapro®), Candesartan (Atacand®), Telmisartan (Micardis®), tasosartan, zolarsartan, Teveten (eprosartan mesylate) or olmesartan medoxomil (Benicar).
- 30 ACE inhibitors, AIIRAs and/or morphogens may be formulated with one or more pharmaceutically acceptable carriers (additives) and/or diluents. As described in detail below, such pharmaceutical compositions may be specially formulated for

administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intrathecal, 5 intracerebroventricular, intramuscular, or intravenous injection as, for example, a sterile solution or suspension, including administration using a minipump or other mechanical-assisted delivery, such as ALZET osmotic pumps that continuously deliver agents at controlled rates; (3) topical application, for example, as a cream, ointment or spray applied to the skin; or (4) intravaginally or intrarectally, for 10 example, as a pessary, cream or foam. However, in certain embodiments the subject compounds may be simply dissolved or suspended in sterile water. In certain embodiments, the pharmaceutical preparation is non-pyrogenic, i.e., does not elevate the body temperature of a patient.

The phrase 'therapeutically effective amount' as used herein means that 15 amount of a compound, material, or composition comprising a compound of the present invention which is effective for producing some desired therapeutic effect in at least a sub-population of cells in an animal and thereby blocking the biological consequences of that pathway in the treated cells, at a reasonable benefit/risk ratio applicable to any medical treatment.

20 The phrase 'pharmaceutically acceptable' is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

25 The phrase 'pharmaceutically acceptable carrier' as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject compounds from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be 'acceptable' in the sense 30 of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically

acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

As set out above, certain compounds contain a basic functional group, such as amino or alkylamino, and are, thus, capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable acids. The term 'pharmaceutically acceptable salts' in this respect, refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds of the present invention. These salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or by separately reacting a purified compound of the invention in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, for example, Berge et al. (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19).

The pharmaceutically acceptable salts of the subject compounds include the conventional nontoxic salts or quaternary ammonium salts of the compounds, e.g., from non-toxic organic or inorganic acids. For example, such conventional nontoxic salts include those derived from inorganic acids such as hydrochloride, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like; and the salts

prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, palmitic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicyclic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isothionic, and the like.

5        In other cases, compounds contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term ‘pharmaceutically acceptable salts’ in these instances refers to the relatively non-toxic, inorganic and organic base addition salts of compounds of the present invention. These salts can likewise be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically acceptable metal cation, with ammonia, or with a pharmaceutically acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, 10 sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like. (See, for example, Berge et al., *supra*).

20      Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

25      Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

30      Formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral

administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the 5 particular mode of administration. The amount of active ingredient that can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 1 per cent to about ninety-nine percent of active ingredient, preferably from about 5 per cent to about 70 per 10 cent, most preferably from about 10 per cent to about 30 per cent.

Methods of preparing these formulations or compositions include the step of bringing into association a compound of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the 15 present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a 20 suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. A compound of the present invention may also be administered 25 as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as 30 starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone,

sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions that can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active

ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. The active ingredient can also be in microencapsulated form, if appropriate, with one or more of the above-described  
5 excipients.

Liquid dosage forms for oral administration of the compounds of the invention include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as,  
10 for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and  
15 mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending  
20 agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared  
25 by mixing one or more compounds of the invention with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound.

Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of a compound of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants that may be required.

The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to a compound of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the compound in a polymer matrix or gel.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise one or more compounds of the invention in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents that delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsulated matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions that are compatible with body tissue.

When the compounds of the present invention are administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

Morphogens, morphogen inducers, or agonists of morphogen receptors, as well as ACE inhibitors (ACEIs) may be administered by any route which is compatible with the particular morphogen, inducer, agonist, or ACEI employed. Thus, as appropriate, administration may be oral or parenteral, including intravenous and intraperitoneal routes of administration. In addition, administration may be by periodic injections of a bolus of the morphogen, inducer, agonist or ACEI, or may be made more continuous by intravenous or intraperitoneal administration from a reservoir which is external (*e.g.*, an ix. bag) or internal (*e.g.*, a bioerodible implant, or a colony of implanted, morphogen-producing cells).

Therapeutic agents of the invention (*i.e.*, morphogens, morphogen inducers, agonists of morphogen receptors, or ACEI) may be provided to an individual by any suitable means, directly (*e.g.*, locally, as by injection, implantation or topical administration to a tissue locus) or systemically (*e.g.*, parenterally or orally). Where the agent is to be provided parenterally, such as by intravenous, subcutaneous, intramolecular, ophthalmic, intraperitoneal, intramuscular, buccal, rectal, vaginal, intraorbital, intracerebral, intracranial, intraspinal, intraventricular, intrathecal, intracisternal, intracapsular, intranasal or by aerosol administration, the agent preferably comprises part of an aqueous or physiologically compatible fluid suspension or solution. Thus, the carrier or vehicle for the agent(s) is physiologically acceptable so that in addition to delivery of the desired agent(s) to the patient, it does not otherwise adversely affect the patient's electrolyte and/or volume balance. The fluid medium for the agent thus can comprise normal physiologic saline (*e.g.*, 9.85% aqueous NaCl, 0.15 M, pH 7-7.4).

Association of the mature morphogen dimer with a morphogen pro domain results in the pro form of the morphogen which typically is more soluble in physiological solutions than the corresponding mature form. In fact, endogenous

morphogens are thought to be transported (*e.g.*, secreted and circulated) in the mammalian body in this form. This soluble form of the protein can be obtained from culture medium of morphogen-secreting mammalian cells, *e.g.*, cells transfected with nucleic acid encoding and competent to express the morphogen. Alternatively,  
5 a soluble species can be formulated by complexing the mature, morphogenically active polypeptide dimer (or an active fragment thereof) with a morphogen pro domain polypeptide or a solubility-enhancing fragment thereof. Solubility-enhancing pro domain fragments can be any N-terminal, C-terminal or internal fragment of the pro region of a member of the morphogen family that complexes  
10 with the mature polypeptide dimer to enhance stability and/or dissolubility of the resulting noncovalent or covalent complex. Typically, useful fragments are those cleaved at the proteolytic site Arg-Xaa-Xaa-Arg (SEQ ID NO: 30). A detailed description of soluble complex forms of morphogenic proteins, including how to make, test and use them, is described in WO 94/03600 (PCT US 93/07189). In the  
15 case of OP-1, useful pro domain polypeptide fragments include the intact pro domain polypeptide (residues 30-292) and fragments 48-292 and 158-292, all of SEQ ID No. 3. Another molecule capable of enhancing solubility and particularly useful for oral administrations, is casein. For example, addition of 0.2% casein increases solubility of the mature active form of OP-1 by 80%. Other components  
20 found in milk and/or various serum proteins may also be useful.

Useful solutions for parenteral administration may be prepared by any of the methods well known in the pharmaceutical art, described, for example, in REMMINGTON'S PHARMACEUTICAL SCIENCES (Gennaro, A., ed.), Mack Pub., 1990. Formulations of the therapeutic agents of the invention may include, for  
25 example, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes, and the like. Formulations for direct administration, in particular, may include glycerol and other compositions of high viscosity to help maintain the agent at the desired locus. Biocompatible, preferably bioresorbable, polymers, including, for example, hyaluronic acid, collagen, tricalcium phosphate,  
30 polybutyrate, lactide, and glycolide polymers and lactide/glycolide copolymers, may be useful excipients to control the release of the agent *in vivo*. Other potentially useful parenteral delivery systems for these agents include ethylene-vinyl acetate

copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation administration contain as excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene 9-lauryl ether, glycocholate and deoxycholate, or oily solutions for administration in  
5 the form of nasal drops, or as a gel to be applied intranasally. Formulations for parenteral administration may also include glycocholate for buccal administration, methoxysalicylate for rectal administration, or citric acid for vaginal administration. Suppositories for rectal administration may also be prepared by mixing the morphogen, inducer or agonist with a non-irritating excipient such as cocoa butter or  
10 other compositions which are solid at room temperature and liquid at body temperatures.

Formulations for topical administration to the skin surface may be prepared by dispersing the morphogen, inducer, agonist, or ACEI with a dermatologically acceptable carrier such as a lotion, cream, ointment or soap. Particularly useful are  
15 carriers capable of forming a film or layer over the skin to localize application and inhibit removal. For topical administration to internal tissue surfaces, the agent may be dispersed in a liquid tissue adhesive or other substance known to enhance adsorption to a tissue surface. For example, hydroxypropylcellulose or fibrinogen/thrombin solutions may be used to advantage. Alternatively, tissue-  
20 coating solutions, such as pectin-containing formulations may be used.

Alternatively, the agents described herein may be administered orally. Oral administration of proteins as therapeutics generally is not practiced, as most proteins are readily degraded by digestive enzymes and acids in the mammalian digestive system before they can be absorbed into the bloodstream. However, the morphogens described herein typically are acid stable and protease-resistant (see, for example, U.S. Pat. No. 4,968,590). In addition, at least one morphogen, OP-1, has been identified in mammary gland extract, colostrum and 57-day milk. Moreover, the OP-1 purified from mammary gland extract is morphogenically active and is also detected in the bloodstream. Maternal administration, via ingested milk, may be a  
25 natural delivery route of TGF- $\beta$  superfamily proteins. Letterio, et al., Science 264:  
30 1936-1938 (1994), report that TGF- $\beta$  is present in murine milk, and that radio-

labeled TGF- $\beta$  is absorbed by gastrointestinal mucosa of suckling juveniles. Labeled, ingested TGF- $\beta$  appears rapidly in intact form in the juveniles' body tissues, including lung, heart and liver. Finally, soluble form morphogen, e.g., mature morphogen associated with the pro domain, is morphogenically active. These 5 findings, as well as those disclosed in the examples below, indicate that oral and parenteral administration are viable means for administering TGF- $\beta$  superfamily proteins, including the morphogens, to an individual. In addition, while the mature forms of certain morphogens described herein typically are sparingly soluble, the morphogen form found in milk (and mammary gland extract and colostrum) is 10 readily soluble, probably by association of the mature, morphogenically active form with part or all of the pro domain of the expressed, full length polypeptide sequence and/or by association with one or more milk components. Accordingly, the compounds provided herein may also be associated with molecules capable of enhancing their solubility in vitro or in vivo.

15 Most ACE inhibitors can be orally administered. In a preferred embodiment, ACE inhibitor as a pharmaceutical composition can be orally administered through drinking water or other suitable liquid carrier.

The compounds provided herein may also be associated with molecules capable of targeting the morphogen, inducer, agonist, or ACEI to the desired tissue. 20 For example, an antibody, antibody fragment, or other binding protein that interacts specifically with a surface molecule on cells of the desired tissue, may be used. Useful targeting molecules may be designed, for example, using the single chain binding site technology disclosed in U.S. Pat. No. 5,091,513. Targeting molecules can be covalently or non-covalently associated with the morphogen, inducer, 25 agonist, or ACEI.

As will be appreciated by one of ordinary skill in the art, the formulated compositions contain therapeutically effective amounts of the morphogen, morphogen inducers, agonists of morphogen receptors, or ACEI. That is, they contain an amount which provides appropriate concentrations of the agent to the 30 affected tissue for a time sufficient to stimulate a detectable restoration of impaired renal system function, up to and including a complete restoration thereof. As will be

appreciated by those skilled in the art, these concentrations will vary depending upon a number of factors, including the biological efficacy of the selected agent, the chemical characteristics (e.g., hydrophobicity) of the specific agent, the formulation thereof, including a mixture with one or more excipients, the administration route,  
5 and the treatment envisioned, including whether the active ingredient will be administered directly into a tissue site, or whether it will be administered systemically. The preferred dosage to be administered is also likely to depend on variables such as the condition of the diseased or damaged tissues, and the overall health status of the particular mammal. As a general matter, single, daily, biweekly  
10 or weekly dosages of 0.00001-1000 mg of a morphogen are sufficient, with 0.0001-100 mg being preferable, and 0.001 to 10 mg being even more preferable.  
Alternatively, a single, daily, biweekly or weekly dosage of about 0.01-1000 µg/kg body weight, more preferably about 0.01-10 µg/kg body weight, or about 10-300 µg/kg body weight may be advantageously employed. As a general matter, single,  
15 daily, biweekly, or weekly dosages of ACEI can be administered orally at an amount of about 0.01 – 300 mg/kg body weight, preferably 0.1-30 mg/kg BW, 0.1-3 mg/kg BW, 1-30 mg/kg BW, most preferably about 1-3 mg/kg BW, in, for example, drinking water, are appropriate for ACE inhibitors. The concentrations can be accordingly adjusted or alternatively expressed as the amount of drug that needs to  
20 be administered per day per kg of body weight, if other factors (such as the average body weight of a subject mammalian patient being treated, and the average amount of water consumed per day by said specific mammalian patient) are provided. The present effective dose can be administered in a single dose or in a plurality (two or more) of installment doses, as desired or considered appropriate under the specific  
25 circumstances. A bolus injection or diffusible infusion formulation can be used. If desired to facilitate repeated or frequent infusions, implantation of a semi-permanent stent (e.g., intravenous, intraperitoneal, intracisternal or intracapsular) may be advisable. It should also be understood that the dosages of morphogens and/or ACEIs, when conjointly administered, may be different from the dosages of  
30 morphogens or ACEIs when they are administered alone (not conjoint administration). It should also be understood that a particular dosage of morphogen or ACEI for treating / preventing chronic renal failure may be different from dosages

used for other non-related (such as bone morphogenesis, etc.) uses of the morphogens and/or ACEIs.

The morphogens, inducers, agonists, or ACEI of the invention may, of course, be administered alone or in combination with other molecules known to be  
5 beneficial in the treatment of the conditions described herein. For example, various well-known growth factors, hormones, enzymes, therapeutic compositions, antibiotics, or other bioactive agents can also be administered with the morphogen and ACEI. Thus, various known growth factors such as NGF, EGF, PDGF, IGF,  
10 FGF, TGF- $\alpha$ , and TGF- $\beta$ , as well as enzymes, enzyme inhibitors, antioxidants, anti-inflammatory agents, free radical scavenging agents, antibiotics and/or chemoattractant / chemotactic factors, can be included in the present morphogen and ACEI formulation.

Finally, it should be understood that morphogen formulation and ACEI formulations of the present invention can be either formulated together, in a single  
15 pharmaceutical composition, or formulated separately, in two or more pharmaceutical compositions. It should also be understood that the same formulation can be administered through different routes, depending on specific needs or appropriate treatment conditions.

*C. Types of Chronic Renal Failures*

20 Many types of Chronic Renal Failure diseases can be treated according to the instant invention. The following discussions are for illustration purpose only, and should not be construed to be limiting in any respect.

The present invention is directed to methods of prevention and/or treatment, and pharmaceutical preparations for use in the prevention and/or treatment, of  
25 vertebrate subjects (preferably mammalian subjects) in, or at risk of, chronic renal failure, or at risk of the need for renal replacement therapy. Such subjects include subjects already afflicted with chronic renal failure, or which have already received renal replacement therapy, as well as any subject reasonably expected to suffer a progressive loss of renal function associated with progressive loss of functioning  
30 nephron units. Whether a particular subject is at risk is a determination which may

routinely be made by one of ordinary skill in the relevant medical or veterinary art. Subjects in, or at risk of, chronic renal failure, or at risk of the need for renal replacement therapy, include but are not limited to the following: subjects which may be regarded as afflicted with chronic renal failure (CRF), end-stage renal disease (ESRD), chronic diabetic nephropathy, diabetic glomerulopathy, diabetic renal hypertrophy, hypertensive nephrosclerosis, hypertensive glomerulosclerosis, chronic glomerulonephritis, hereditary nephritis, or renal dysplasia; subjects having a biopsy indicating glomerular hypertrophy, tubular hypertrophy, chronic glomerulosclerosis, and/or chronic tubulointerstitial sclerosis; subjects having an ultrasound, MRI, CAT scan, or other non-invasive examination indicating renal fibrosis; subject shaving an unusual number of broad casts present in urinary sediment; subjects having a GFR which is chronically less than about 50%, and more particularly less than about 40%, 30% or 20%, of the expected GFR for the subject; human male subjects weighing at least about 50 kg and having a GFR which is chronically less than about 50 ml/min, and more particularly less than about 40 ml/min, 30 ml/min or 20 ml/min; human female subjects weighing at least about 40 kg and having a GFR which is chronically less than about 40 mL/min, and more particularly less than about 30 mL/min, 20 ml/min or 10 ml/min; subjects possessing a number of functional nephron units which is less than about 50%, and more particularly less than about 40%, 30% or 20%, of the number of functional nephron units possessed by a healthy but otherwise similar subject; subjects which have a single kidney; and subjects which are kidney transplant recipients.

Chronic renal failure (CRF) can be classified by the site (location) of primary damage: Pre-renal CRF, Post-renal CRF and Renal CRF.

25        Pre-Renal CRF. Some medical conditions cause continuous hypoperfusion (low blood flow) of the kidneys, leading to kidney atrophy (shrinking), loss of nephron function, and chronic renal failure (CRF). These conditions include poor cardiac function, chronic liver failure, and atherosclerosis ("hardening") of the renal arteries. Each of these conditions can induce ischemic nephropathy, which is a result 30 of inadequate blood flow (hypoperfusion) to the kidneys. Hypoperfusion manifests

as a progressive loss of kidney function and kidney atrophy (shrinkage). Renal failure results when this process damages both kidneys.

Post-Renal CRF. Interference with the normal flow of urine can produce backpressure within the kidneys, can damage nephrons, and lead to obstructive 5 uropathy, a disease of the urinary tract. Abnormalities that may hamper urine flow and cause post-renal CRF include the following:

- **Bladder outlet obstruction** due to an enlarged prostate gland or bladder stone;
- **Neurogenic bladder**, an over distended bladder caused by impaired 10 communicator nerve fibers from the bladder to the spinal cord;
- **Kidney stones** in both ureters, the tubes that pass urine from each kidney to the bladder;
- **Obstruction of the tubules**, the end channels of the renal nephrons;
- **Retroperitoneal fibrosis**, the formation of fiber-like tissue behind the 15 peritoneum, the membrane that lines the abdominal cavity;
- **Vesicoureteral reflux (VUR)**, the backward flow of urine from the bladder into a ureter.

Renal CRF. Chronic renal failure caused by changes within the kidneys, is called renal CRF, and is broadly categorized as follows:

- **Diabetic nephropathy**, kidney disease associated with diabetes; the most common cause of CRF;
- **Hypertension Nephrosclerosis**, a condition that occurs with increased frequency in African Americans; the second leading cause of CRF;
- **Chronic glomerular nephritis**, a condition caused by diseases that affect 25 the glomeruli and bring about progressive dysfunction;
- **Chronic interstitial nephritis**, a condition caused by disorders that ultimately lead to progressive scarring of the interstitium;

- **Renal vascular CRF**, large vessel abnormalities such as renal artery stenosis (narrowing of the large arteries that supply the kidneys);
- **Vasculitis**, inflammation of the small blood vessels;
- **Cystic kidney disease**, kidney disease distinguished by multiple cysts (lined cavities or sacs);
- **Hereditary diseases of the kidney**, such as Alport's syndrome (hereditary nephritis).

More detailed descriptions of a few of the above conditions are provided below.

10        Diabetic nephropathy is kidney disease that develops as a result of diabetes mellitus (DM). DM, also called simply diabetes, affects approximately 5% of the U.S. population. This disease damages many organs, including the eyes, nerves, blood vessels, heart, and kidneys. DM is the most common cause of kidney failure in the United States and accounts for over one-third of all patients who are on  
15        dialysis.

DM patients are unable to metabolize carbohydrates (e.g., food starches, sugars, cellulose) properly. The disease is characterized by excessive amounts of sugar in the blood (hyperglycemia) and urine; inadequate production and/or utilization of insulin; and by thirst, hunger, and loss of weight.

20        Diabetics who require daily insulin shots to maintain life have *insulin-dependent diabetes mellitus*, or DM 1. In this type of diabetes, the pancreas  $\beta$  cells secrete little or no insulin and the blood sugar level remains high, unless treated. DM 1 usually occurs in children and young adults and is often called juvenile onset diabetes. Onset of the disease is abrupt. The patient becomes very sick and requires  
25        immediate insulin therapy. Approximately 1 million people in the United States have DM 1.

Approximately 25% to 40% of patients with DM 1 ultimately develop diabetic nephropathy (DN), which progresses through about five predictable stages. During Stage 1 (very early diabetes), increased demand upon the kidneys is  
30        indicated by an above-normal glomerular filtration rate (GFR). During Stage 2

- (developing diabetes), the GFR remains elevated or has returned to normal, but glomerular damage has progressed to significant microalbuminuria (small but above-normal level of the protein albumin in the urine). Patients in stage 2 excrete more than 30 mg of albumin in the urine over a 24-hour period. Significant
- 5       microalbuminuria will progress to end-stage renal disease (ESRD). Therefore, all diabetes patients should be screened for microalbuminuria on a routine (yearly) basis. During Stage 3 (overt, or dipstick-positive diabetes), glomerular damage has progressed to clinical albuminuria. The urine is "dipstick positive," containing more than 300 mg of albumin in a 24-hour period. Hypertension (high blood pressure)
- 10      typically develops during stage 3. During Stage 4 (late-stage diabetes), glomerular damage continues, with increasing amounts of protein albumin in the urine. The kidneys' filtering ability has begun to decline steadily, and blood urea nitrogen (BUN) and creatinine (Cr) has begun to increase. The glomerular filtration rate (GFR) decreases about 10% annually. Almost all patients have hypertension at stage
- 15      4. During Stage 5 (end-stage renal disease, ESRD), GFR has fallen to approximately 10 milliliters per minute (<10 mL/min) and renal replacement therapy (i.e., hemodialysis, peritoneal dialysis, kidney transplantation) is needed. Progression through these five stages is rather predictable because the onset of DM 1 can be identified, and most patients are free from age-related medical problems.
- 20      *Non-insulin-dependent diabetes*, or DM 2, differs from DM 1 in that the main problem is a peripheral resistance to the action of the insulin. DM 2 usually occurs in adults over the age of 40 who are overweight and have a family history of the disease. Some patients can manage their diabetes with weight loss and changes in their diet. Others require medication, and many with DM 2 eventually require
- 25      insulin. Onset is gradual, and patients may be sick for quite some time without knowing it. Nearly 95% of diabetics are diagnosed with DM 2. An estimated 5% to 15% of DM 2 cases also progress through the five stages of diabetic nephropathy (DN), but the timeline is not as clear. Some patients advance through the stages very quickly.
- 30      Renal artery stenosis (RAS) is the narrowing of the lining of the main artery that supplies the kidney. Most RAS is caused by atherosclerosis or "hardening of the

arteries." Atherosclerosis is the build up of cholesterol deposits, or plaque, in the lining of the arteries. Depending on the degree of narrowing, patients can develop hypertension called renal vascular hypertension (RVH). This form of hypertension is the most common cause of secondary hypertension. In fact, hypertension is second  
5 only to diabetes as the leading cause of kidney failure. In the US, 15%-20% of kidney failure cases are due to hypertension. RVH occurs when RAS produces a critical narrowing of the artery that supplies one of the kidneys. Critical RAS is defined as at least 70% narrowing of the renal artery, based on angiographic (blood vessel x-ray) evaluation. Reduced blood flow through the renal artery causes the  
10 kidney to release increased amounts of the hormone renin. Renin, a powerful blood pressure regulator, initiates a series of chemical events that result in hypertension. Renal vascular hypertension can be very severe and difficult to control.

The kidney with RAS suffers from the decreased blood flow and often shrinks in size (atrophies). This process is called ischemic nephropathy. The other  
15 kidney is at risk for developing damage from the hypertension, often developing hypertensive nephrosclerosis. The persistent elevated blood pressures in this non-stenotic kidney can cause progressive scarring (sclerosis) leading to progressive loss of filtering function in this kidney as well. Both unilateral RAS and bilateral RAS can ultimately lead to chronic renal failure.

20 There are two types of RAS: Atherosclerotic Renal Artery Stenosis (AS-RAS) and Fibromuscular Dysplasia (FMD). AS-RAS is due to the build-up of cholesterol on the inner lining of the renal artery. It is exceedingly more common than the unusual case of FMD-RAS. FMD-RAS occurs almost exclusively in women aged 30 to 40 and rarely affects African Americans or Asians. FMD-RAS is  
25 due to an abnormality in the muscular lining of the renal artery. There is often a familial history of FMD RAS.

Cystic kidney disease describes several conditions in which fluid-filled cysts form in the kidneys. Cysts generally develop in weak segments of the tubules that carry urine from the glomeruli. The cyst's growth displaces healthy kidney tissue.  
30 The kidneys expand to accommodate the cyst, which can weigh as much as 20 pounds. Three factors determine cyst classification: its cause (acquired, inherited),

its features (complicated, simple, multiple, single), and its location (outer [cortical] or inner [medullary] kidney tissue).

Polycystic kidney disease (PKD; common, with several cysts in the kidney) is a primary cystic kidney disease. PKD type 1 and PKD type 2 are caused by 5 autosomal dominant mutations on chromosomes 16 and 4, respectively, and run in families. PKD autosomal recessive has been linked to chromosome 6. Polycystic kidney disease (PKD) is the most frequently inherited disease; it affects approximately 600,000 people in the United States and over 12,000,000 worldwide. Most suffer from the autosomal dominant type. It is the fourth leading cause of 10 kidney failure and causes 10% of all end-stage renal disease (ESRD), usually between the ages of 40 and 60. It affects men, women, and races equally.

Secondary cystic kidney disease include Acquired cystic kidney disease (ACKD); Medullary cystic disease (inner kidney), which includes Juvenile nephronophthisis (during adolescence) and Medullary sponge kidney (deterioration 15 of kidney with cysts); and Renal cell cancer associated cysts.

Autosomal dominant medullary cystic kidney disease (MCK) causes cysts to form in the inner tissue of the kidney and can develop at a very early age. Recessive juvenile nephronophthisis usually occurs later than MCK, but is associated with similar symptoms, including chronic renal failure and growth problems. Small cysts 20 in the collecting ducts of the inner kidney characterize medullary sponge kidney (MSK), which is associated with hematuria and kidney stones, but not chronic renal failure (CFR).

Acquired cystic kidney disease (ACKD) affects patients with chronic renal failure and causes hematuria, erythrocytosis (increase in red blood cells), and is 25 associated with the development of cancer. Causes of acquired cystic kidney disease (ACKD) are long-term disease (glomerulonephritis) and the scarring that often results from dialysis. ACKD is common among patients with chronic renal failure (CRF). Nearly all of those who use dialysis for more than 5 years develop ACKD.

30 Proteinuria is an abnormally high amount of protein in the urine. Proteins in the blood, like albumin and immunoglobulin, help coagulation (clotting), balance bodily fluids, and fight infection. The kidneys remove wastes from protein-rich

blood through millions of tiny filtering screens called glomeruli. Most proteins are too large to pass through the glomeruli into the urine. The glomeruli are negatively charged, so they repel the negatively charged proteins. Thus, a size and charge barrier keeps protein molecules from entering the urine. But when the glomeruli are 5 damaged, proteins of various sizes pass through them and are excreted in the urine.

The following five types of proteinuria are distinguished by milligrams (mg) of protein measured during a 24-hour urine collection:

1. Microalbuminuria	30 - 150 mg
2. Mild	150 – 500 mg
3. Moderate	500 – 1000 mg
4. Heavy	1000 – 3000 mg
5. Nephrotic range	more than 3500 mg

As kidney disease progresses, more protein enters the urine. People with 10 nephrotic-range proteinuria typically have extensive glomeruli damage and usually develop nephrotic syndrome (see below).

Hypertension and diabetes are the two biggest risk factors for proteinuria. Old age and weight gain also increase the risk. The following conditions cause proteinuria:

- 15      • Acute glomerulonephritis;
- Amyloidosis (protein deposits associated with chronic disease);
- Focal glomerulonephritis;
- Hypertension;
- IgA nephropathy;
- 20      • Mesangial proliferation
- Minimal change disease;

Foamy urine and swelling (edema) are two signs of proteinuria that become more evident as the disease progresses. Excess protein can cause the urine to foam in water. This occurs because protein changes the surface tension between urine and water. Edema usually only occurs in nephrotic range proteinuria.

5       Albumin is particularly useful in absorbing bodily fluid into the blood. Because the albumin molecule is relatively small, it is often among the first proteins to enter the urine after glomeruli are damaged. Therefore, even minor kidney dysfunction is detectable with proper diagnosis of microalbuminuria. Reduced albumin level in the blood causes fluid retention and swelling that is first noticeable  
10      in the hands, lower legs, and feet. In more serious cases, the abdomen and face may swell.

Orthostatic proteinuria is a disorder seen occasionally in children and young adults who leak significant amounts of urine when they are upright (orthostatic). Presumably, standing increases the pressure on the glomeruli and causes more  
15      protein to enter the urine, while lying down relieves pressure and causes less protein leakage. This is a benign disorder that most young people outgrow.

Hypertensive people who develop proteinuria stand a significant chance for kidney failure. African Americans are 20 times more likely than Caucasians to develop hypertensive-related kidney failure. Proteinuria in people with diabetes may  
20      be a sign that kidney disease is worsening. Microalbuminuria is often cited as a risk for coronary artery disease (CAD) and is often diagnostic of it and related cardiovascular conditions.

Nephrotic syndrome (NS) is a condition that is often caused by any of a group of diseases that damage the kidneys' filtering system, the glomeruli. The  
25      structure of the glomeruli prevents most protein from getting filtered through into the urine. Normally, a person loses less than 150 mg of protein in the urine in a 24-hour period. Nephrotic-range proteinuria, the urination of more than 3.5 grams of protein during a 24-hour period, or 25 times the normal amount, is the primary indicator of NS.

30       About two in every 10,000 people experience nephrotic syndrome. Nephrotic syndrome prevalence is difficult to establish in adults because the condition is

usually a result of an underlying disease. In children, it is diagnosed in more boys than girls, usually between 2 and 3 years of age.

In addition to proteinuria, there are three main symptoms of nephrotic syndrome associated with protein leaking into the urine:

- 5     • Hypoalbuminemia (low level of albumin in the blood);
- Edema (swelling);
- Hypercholesterolemia (high level of cholesterol in the blood);

10     *Hypoalbuminemia* is a low level of albumin (a protein) in the blood due to proteinuria. Low albumin in the blood causes fluid to move from the blood into the tissue, causing swelling. The kidney perceives the decrease of fluid in the blood and aggressively retains as much fluid and salt as it can. This contributes to the body's fluid-overload state.

15     Nephrotic-related swelling makes tissue puffy, soft, and impressionable to the touch. Edema is most common in the legs and feet, especially after standing all day. It can cause feelings of tightness in the extremities and may affect mobility. In later stages, swelling may occur in the abdomen (ascites), hands, and around the eyes in the morning (called periorbital edema). In later stages, the whole body may swell (anasarca). Some people gain weight after fluid builds up in their bodies for a long time.

20     *Hypercholesterolemia*, high blood cholesterol, is common in nephrotic syndrome. In addition to albumin, other important enzymes involved in cholesterol metabolism slip through the glomeruli, which contribute to high blood cholesterol.

25     Nephrotic syndrome is associated with renal failure. The disease that causes NS can damage the glomeruli and can interfere with their ability to clean the blood. The edema that is present in the legs may also be occurring in the kidney tissue itself and can interfere with the kidneys' ability to clean the blood. Renal failure can either be gradual (CRF) or acute (ARF).

A hypercoaguable state, in which the blood abnormally overclots, is also seen in some patients with NS. This means that they are at risk for developing a

blood clot in the legs or in the renal veins that transport blood from the kidney.

Some patients take blood thinners to prevent this complication.

There are a number of different disorders that can cause NS. Diabetes and, to a lesser extent, hypertension can cause diffuse damage to the glomeruli and can

5 ultimately lead to NS. The following diseases can cause specific damage to the glomeruli and often result in the development of heavy proteinuria and in many instances NS: Amyloidosis (the stiffening and subsequent malfunction of the kidney due to fibrous protein deposit in the tissue); Congenital nephrosis; Focal segmental glomerular sclerosis (FSGS) (creates scar tissue in the glomerulus, damaging its

10 protein-repellant membrane); Glomerulonephritis (GN), including Diffuse mesangial proliferative GN (affecting the messangium), Membranous (damages the protein-repellant membrane), and Post infectious (occurs after an infection); IgA nephropathy (Berger's disease) (deposit of specific immunoglobulin A causing an inflammatory reaction and leading to glomerulonephritis); Minimal change disease

15 (Nil's disease); and Pre-eclampsia (rarely associated with NS, more often associated with heavy proteinuria).

Many of these diseases tend to occur more often in certain age groups. Less than 1yr old: Congenital nephrosis. Less than 15 years old: Min change, FSGS, and Other. Age 15 to 40: Min change, FSGS, and Other. Over age 40: Membranous GN,

20 and Diabetic nephropathy. Over 60: Amyloidosis may account for up to 20% of cases.

In addition, physical injury to the kidneys (loss of one kidney plus damage to the other, etc.) and other equivalent situations, such as complete or partial loss of kidney function due to diseases affecting the total number of functional nephrons

25 (filtering units that consist of a glomerulus and corresponding tubule), may cause the remaining functional nephrons to "attempt" compensating for the renal damage by hyperfiltration (excessive straining of the blood). Over time, hyperfiltration causes further loss of renal function, leading to chronic renal failure.

#### **IV. Exemplification**

Practice of the invention, including additional preferred aspects and embodiments thereof, will be still more fully understood from the following examples, which are presented herein for illustration only and should not be construed as limiting the invention in any way.

5 For the following experiments, "OP-1" is used interchangably with "sBMP-7."

*Example 1 Nephrectomy Chronic Renal Failure Injury Model*

A rat partial (5/6) nephrectomy or rat remnant kidney model (RRKM) model was employed essentially as described (Vukicevic, et al. (1987) J. Bone Mineral Res. 2: 533, the entirecontent of which is incorporated herein by reference). Male Munich-Wistar rats (2-3 months old, weighing about 200-250 g) were subjected to renal mass ablation. Specifically, the right kidney is excised in conjunction with selective ligation of the left renal artery branches such that one third of the kidney remains perfused (5/6 NPX, see Figure 12). Immediately following surgery, plasma creatinine and BUN levels rise dramatically due to the loss of renal mass and function. Over the next several weeks of this "acute" failure phase, plasma creatinine and BUN levels of surviving animals decline somewhat toward normal values but remain elevated. Renal function then appears to remain relatively constant or stable for a period of variable duration. After this point, the animals enter a period of chronic renal failure in which there is an essentially linear decline in renal function ending in death. For these reasons, animals are given 4 weeks of recovery prior to initiation of treatments.

Four weeks after nephrectomy, rats were divided into four treatment groups (see Table III), namely OP-1 (150 µg/kg body weight, 3X/week, by i.p. injection),  
25 Vehicle (20 mM arginine/150 mM NaCl, 0.1% Tween-80, pH 9.0, 1 ml/kg body weight, 3X/week, by i.p. injection), enalapril (100 mg/L in drinking water, 8-16 mg /kg body weight), and OP-1 with enalapril. For the first two groups (OP-1 and Vehicle), animals were sacrificed 14 weeks after nephrectomy. For the last two groups (enalapril, and OP-1 with enalapril), animals were sacrificed 26 weeks after 30 nephrectomy. As surgical controls, rats were subjected to a "sham" operation in which the kidneys were decapsulated but no renal tissue was removed. Sham-treated

animals were sacrificed 26 weeks after operation. None of the rats died in any group during this study. Systolic blood pressure, urine protein and/or glomerulosclerosis were monitored at intervals between 4 and 26 weeks post surgery.

**Table III. Experimental design in the Nephrectomy Chronic Renal Failure**

5

**Injury Model.**

Group	Animals (N)	Treatment	Duration
I	5	Sham	26 weeks
II	10	OP-1	Initiate treatment 4 weeks after nephrectomy Terminate animals 14 weeks after nephrectomy
III	10	Vehicle	
IV	10	OP-1 + enalapril	Initiate treatment 4 weeks after nephrectomy Terminate animals 26 weeks after nephrectomy
V	10	Vehicle + enalapril	

As compared to sham-operated controls, animals after Nephrectomy exhibited symptoms of higher blood pressure and higher proteinuria. While OP-1 did not dramatically lower the blood pressure level (see Figure 13), OP-1 significantly reduced the proteinuria level, from 91 mg/day to 71 mg/day ( $P < 0.05$ ), after 14 weeks post-Nephrectomy (see Figure 14). When animals were co-treated with OP-1 and enalapril, there was no additional benefit in reducing the blood pressure of nephrectomized animals to normal level as compared to animals treated by the ACE inhibitor enalapril alone (see Figure 15). However, the combination of OP-1 and enalapril was more effective in reducing the proteinuria level than enalapril alone, 62 mg/day vs. 105 mg/day (see Figure 16).

In summary, the results from the Nephrectomy Chronic Renal Failure Injury Model demonstrate that OP-1 improves glomerular filtration rate, reduces glomerulosclerosis, and reduces proteinuria. Co-treatment of OP-1 and enalapril reduces late-stage proteinuria more than enalapril alone, thus may have a better effect on renal functions.

*Example 2      Unilateral Ureteral Obstruction (UUO) Renal Fibrosis Model*

This UUO model was employed essentially as described (Moller, et al. (1984) *Virchows Arch* 402: 209-237, the entire contents of which are incorporated herein by reference). Sprague-Dawley rats (about 250 g) underwent either a sham operation (ureter manipulated but not ligated) or unilateral ureteral ligation. Two 5 ligatures, 5 mm apart, were placed in the upper two-thirds of the ureter over a section of polyethylene tubing placed around the ureter (see Figure 17). The suture tied to obstruct the ureter was removed along with the tubing at day 5, relieving the obstruction. In this model, hypertension, proteinuria, and lipid dysregulation do not contribute to progressive nephron destruction, and glomerular injury is not prominent 10 early in the course of the injury produced. Uremia is avoided by the function of the contralateral kidney, which undergoes hypertrophy and hyperplasia as the obstructed kidney is destroyed. The renal injury of UUO is mediated in part through stimulation of renal angiotensin II production, which activates type-1 A-II Receptor and the downstream TGF- $\beta$  in a cascade of events culminating in tubulointerstitial 15 inflammation and fibrosis. Inhibition of angiotensin II production by ACE inhibitors (or inhibition of A-II receptors) decreases expansion of the renal interstitium associated with fibrosis.

The effect of OP-1 on Renal fibrosis has been published (Hruska, et al. (2000) *Am J Physiol Renal Physiol* 279:F130-43, incorporated herein by reference), 20 and a summarization is as follows. Administration of OP-1 (100 or 300  $\mu$ g/kg body weight) prevented interstitial inflammation and fibrogenesis during the first 5 days after obstruction. Compared with ACE inhibition (by enalapril treatment), OP-1 was more effective in preventing tubulointerstitial fibrosis and in preserving renal 25 function (see Figure 18). The mechanism of OP-1- induced renal protection was associated with prevention of tubular atrophy, an effect not shared with enalapril (see Figure 19). OP-1 blocked the stimulation of epithelial cell apoptosis produced by UUO, which promoted maintenance of tubular epithelial integrity. OP-1 preserved renal blood flow (RBF) during UUO, but enalapril also stimulated RBF. OP-1 was more efficacious than enalapril in improving the glomerular filtration rate 30 as evidenced by the inulin clearance rate (see Figure 20). OP-1 also inhibited tubular epithelial disruption stimulated by the renal injury of UUO. Additional effects of OP-1 have been observed in this rat UUO model. For example, OP-1, but not ACE

inhibitor, significantly reduced the loss of medullary tissue in the kidney (see Figure 21), from about 24% to about 16% (OP-1 at 100 µg/kg) or about 13% (OP-1 at 300 µg/kg) (P <0.01).

In summary, the results from the UUO Renal Fibrosis Model demonstrate  
5 that OP-1 reduces renal fibrosis, increases the glomerular filtration rate, and reduces tubular atrophy and medullary necrosis. OP-1 is more effective than enalapril. However, the combination of OP-1 and enalapril may have additive benefits on renal functions.

*Example 3 Streptozotocin-Induced Diabetic Nephropathy Model*

10 Nephropathy is one of the most common and most serious complications in type 1 diabetes mellitus. Renal involvement usually starts with renal hypertrophy and glomerular hyperfiltration, which can be observed soon after diabetes onset (Mogensen, et al. (1994) Diabetes Care 17:770-775). Glomerular hyperfiltration is often accompanied by a loss of renal functional reserve. After some years,  
15 microalbuminuria (30 to 300 mg/day) may occur as well as morphological changes such as thickening of the glomerular basement membrane and mesangial expansion. The albumin leakage may subsequently become aggravated and overt nephropathy with albuminuria (>300 mg/day) may develop, usually 10 to 20 years after the onset of diabetes. At this time, hypertension becomes more common. Nephrotic syndrome  
20 may occur, and glomerular filtration rate declines. The most important therapeutic measures undertaken to avoid, or retard, the progress of nephropathy aim to improve glycemic control and normalize blood pressure. ACE inhibitors have proven effective in the latter respect.

Streptozotocin kills pancreatic β cells and induces type I diabetes (for  
25 review, see Cheta et al. (1998) J Pediatr Endocrinol Metab 11:11-9). It is widely used to induce experimental diabetic nephropathy in animals (see Figure 22). Adult female Sprague-Dawley rats (weighing 200-250 g) were intraperitoneally injected with streptozotocin (60 mg/kg body weight) to induce hyperglycemia. Hyperglycemic rats then received daily injections of insulin to maintain blood  
30 glucose between 200-400 mg/dL. At week 16 when renal function declined, animals were treated weekly with OP-1 (10, 30 or 100 µg/kg body weight), enalapril (50 or

100 mg/L in drinking water) or a combination of OP-1 and enalapril. Control animals without streptozotocin treatment were handled in all other ways like treated animals. Animals were sacrificed at week 32 post-streptozotocin treatment.

5 Animals treated with streptozotocin exhibited symptoms of lower glomerular filtration rate and higher proteinuria level. As seen in Figure 23, OP-1, but not enalapril, significantly increased the glomerular filtration rate (GFR). While the mean GFR in the 32-week diabetic animals was about 0.38 ml/min/100 g body weight, the mean GFR in the animals treated with 100 µg/kg of OP-1 was about 0.7 ml/min/100 g body weight ( $P<0.05$ ). The mean GFR in the animals co-treated with 10 OP-1 and enalapril was even higher, about 0.75 ml/min/100 g body weight ( $P<0.05$ ). As seen in Figure 24, OP-1 (30 and 100 µg/kg), enalapril (50 and 100 mg/L) or the combination of OP-1 and enalapril, significantly reduced the proteinuria level from about 140 mg/day to almost normal level.

15 In summary, the results from Streptozotocin-Induced Diabetic Nephropathy Model demonstrate that OP-1 increases the glomerular filtration rate and reduces proteinuria. The combinatorial treatment of OP-1 and enalapril may have better effects on renal functions.

*Example 4 Alloxan-Induced Diabetic Nephropathy Model*

Similar to streptozotocin, alloxan kills pancreatic  $\beta$  cells and induces type I diabetes (for review, see Cheta et al. (1998) J Pediatr Endocrinol Metab 11:11-9). Alloxan is also widely used to induce experimental diabetic nephropathy in animals (Figure 25). Adult female Sprague-Dawley rats (weighing 200-250 g) were intraperitoneally injected with alloxan (70 mg/kg body weight) to induce hyperglycemia. Renal arteries were clamped just prior to injection to prevent direct nephrotoxicity, and then clamps were removed 5 minutes after injection. At week 16 when renal function declined, animals were treated with OP-1 (10 µg/kg body weight, 3X/week, or 30 µg/kg body weight, 1X/week), enalapril (100 mg/L in drinking water) or the combination of OP-1 and enalapril. Control animals without alloxan treatment were handled in all other ways like treated animals. The treatment 25 duration was 12 weeks. All rats were sacrificed after 26 weeks.

Animals treated with alloxan showed higher serum creatinine level and higher proteinuria after 12-weeks of treatment. As seen in Figure 26, OP-1 (10 or 30 µg/kg) dramatically reduced the serum creatinine level, from about 115 µmole/L to about 65 µmole/L or 55 µmole/L ( $P<0.01$ ). Compared with OP-1, elalapril reduced 5 the serum creatinine level at a lesser degree. The combination of OP-1 and elanapril also significantly reduced the serum creatinine level. As seen Figure 27, OP-1 (10 or 30 µg/kg) or enalapril reduced the proteinuria level, from about 180 mg/dL/24 hr to about 80 (or 110) or 140 mg/dL/24 hr, respectively. In contrast, the combination of OP-1 and elanapril dramatically reduced the proteinuria level to as low as about 30 10 mg/dL/24 hr ( $P<0.01$ ).

In summary, the results from Alloxan-Induced Diabetic Nephropathy Model demonstrate that OP-1 or enalapril decreases the serum creatinine level and reduces the proteinuria level. The combinatorial treatment of OP-1 and enalapril shows better effects on renal functions.

15     *Example 4     Bone Morphogenic Protein-7 (BMP-7), a Novel Effective Therapy  
For Diabetic Nephropathy*

Overview

A long-term streptozotocin model of diabetic nephropathy was used to test and compare the therapeutic actions of BMP-7 with those of Enalapril. The study 20 design was a treatment protocol beginning at 16 weeks when glomerular hypertrophy and proteinuria were established. The effects of therapy with BMP-7 (10, 30, or 100 µg/kg iv, biw) were compared to a maximal dose of Enalapril (20 mg/kg) and to a vehicle control. The highest dose of BMP-7 and Enalapril were equal in partially reversing kidney hypertrophy. In the diabetic rats treated with 25 BMP-7, 100 µg/kg, GFR at 32 weeks was significantly higher than in the diabetic vehicle treated rats,  $0.7 \pm 0.08$  vs  $0.34 \pm 0.02$  ml/min/100g bw ( $P < 0.05$ ). The GFR of 32 week diabetic Enalapril treated rats was  $0.58 \pm 0.06$  (not significant compared to vehicle treated and sham injected rats  $0.55 \pm 0.02$ ). Albuminuria was reversed to normal by BMP-7 in a dose dependent manner.

The reduction in proteinuria by the intermediate dose of BMP-7 was similar to the effect of Enalapril therapy. Glomerular area and interstitial volume were significantly decreased in the BMP-7 and Enalapril treated animals. Glomerular sclerosis was prevented by BMP-7 therapy more effectively than by Enalapril. The 5 first insight into the mechanism of BMP-7 therapy, was produced by analysis of blood pressures. Enalapril controlled hypertension throughout the course of therapy while BMP-7 did not effect blood pressure until the final four weeks of therapy. Important mechanistic insight derived from the demonstration that lost epithelial cell differentiation marked by loss at hyperglycemic vehicle treated diabetic rats BMP-7 10 expression in the kidney. At the same time another developmental morphogen, Wnt4, was widely expressed. BMP-7 and Enalapril therapy restored BMP-7 expression at high levels in the collecting duct without affecting Wnt4 expression. We conclude that BMP-7 reversed diabetic and hyperglycemia induced glomerular hypertrophy and injury, restoring GFR, protein excretion in glomerular histology 15 towards normal and generally outperforming Enalapril. Restoration of BMP-7 expression, representing preservation of the collecting duct phenotype, restored the normal developmental Wnt4 interacting partner. This was associated with a successful repair reaction and a reversal of the ill-fated injury response.

#### Introduction

20 Recent studies have made the surprising observation that a renal tubular developmental morphogen, bone morphogenetic protein-7 (BMP-7), was effective in preventing the tubulointerstitial nephritis stimulated by obstructive uropathy (1). The mechanism of action appeared to be preservation of epithelial cell phenotype, inhibition of epithelial-mesenchymal transdifferentiation and inhibition of injury 25 induced epithelial cell apoptosis. These actions of BMP-7 are reminiscent of the effects that the morphogen exercises during development.

During vertebrate development, the permanent kidney is generated by the interactions of the ureteric bud and the metanephric mesenchyme (2,3). At day 11 post-coitum (dpc) in the mouse, the ureteric bud branches out and invades the 30 metanephric mesenchyme. Thereafter, nephrogenesis derives from reciprocal inductive interaction between these two tissues. The metanephric mesenchyme

induces the ureteric bud to grow and bifurcate to form the collecting ducts. At the same time permissive survival signals from the ureteric bud interact with mesenchymal signals to induce the conversion of metanephric mesenchyme into an epithelial structure. Epithelialization begins at day 11.5 pc with condensation around  
5 the ureteric bud, and it progresses with the condensed mesenchyme segregating into pretubular aggregates. Epithelialization of these aggregates leads to development of the comma shaped bodies, S-shaped bodies and eventually the epithelial component of the nephron including glomerular podocytes.

BMP-7 has been shown to be a required factor leading to the condensation  
10 and epithelialization of the metanephric mesenchyme, and the reciprocal induction of collecting duct differentiation (4-6). It is expressed in the ureteric bud, and in the condensing mesenchyme at day 11.5. BMP-7 is a survival factor for the condensing mesenchymal cells which die between 12.5 and 14.5 dpc in its absence (4). At day 12 pc, in the absence of BMP-7, glomerulus formation ceases as the mesenchymal  
15 cells apoptose. BMP-7 interacts with another critical tubular inductive morphogen, Wnt4 (7) (8). Wnt4 expression is initiated at day 12.5 in the aggregating mesenchyme and pretubular aggregates. It is required as an inductive signal for epithelialization (7). At birth, BMP-7 deficient kidneys are dysgenic, hypoplastic and cystic with severely dilated collecting ducts separated by areas of stromal cells and  
20 extracellular matrix. The kidneys are hydronephrotic, and they do not have metanephric mesenchyme or evidence of glomerulus formation in the cortical nephrogenic zone (4,5). Cysts appear to originate from derivatives of the ureteric bud suggesting abnormal activity of the cell cycle and disordered polarity in these cells. Glomerular density is less than 3/ section compared to greater than 100/section  
25 in wild type kidneys (8). Wnt4 deficient kidneys, on the other hand are also dysplastic and hydronephrotic, but they are totally devoid of glomeruli (8).

Compared to many other morphogens, expression of BMP-7 in the tubular epithelial segments derived from the ureteric bud does not cease following its developmental inductive actions, rather its expression persists and it likely functions  
30 physiologically as a collecting duct epithelial cell differentiation factor. As such it

inhibits proliferation by blocking progress of the cell cycle at the G1 checkpoint and it prevents apoptosis (1,9).

Tissue injuries frequently stimulate attempts at repair that recapitulate development, including entry of surviving tissue cells into the cell cycle. This would 5 require the actions of differentiation factors to be overcome perhaps by decreasing their levels. In turn, the absence of key factors could cause these attempted repairs to default into a fibrotic process resulting in permanent loss of the starting tissue. Unsuccessful injury repair characterizes many renal diseases that lead to loss of excretory function. Recently, renal injuries, including that caused by high glucose 10 levels, have been shown to decrease BMP-7 levels (1,10-12). Treatment of one of these injuries with BMP-7 prevented renal tubular atrophy and epithelial cell apoptosis. Failure of tubular development and mesenchymal apoptosis are features of development in the BMP-7 deficient state.

Here we report in a proof of concept study that BMP-7 is an effective 15 therapy for diabetic nephropathy. We report the discovery of a new mechanism of renal injury stimulated by diabetes, that of re-expression of a critical tubular epithelial inductive signal, Wnt4, that interacts with BMP-7 during development. Wnt4 is known to stimulate tubulointerstitial fibrosis, and its re-expression during 20 diabetic hyperglycemic injury is a mechanism of synergism with TGF® resulting in a failed injury repair reaction and promotion of disease. Therapy of diabetic nephropathy with BMP-7 partially reversed the renal injury induced by diabetes and hyperglycemia, and it prevented the development of glomerulosclerosis. The actions of BMP-7 were compared to the known therapeutic agent for diabetic nephropathy – angiotensin converting enzyme inhibition. While both agents were efficacious, 25 BMP-7 was more effective in reversing proteinuria and preventing glomerulosclerosis. Diabetic injury resulted in the loss of tubular epithelial and glomerular podocyte phenotype manifested by loss of BMP-7 expression, and therapy with BMP-7 restored the phenotype of the collecting duct manifested by restoration of BMP-7 expression. BMP-7, in turn, stimulated a successful repair 30 reaction possibly by interacting with Wnt4 and inhibiting the actions of TGF®.

#### Materials and Methods

*Animals:* Female Sprague-Dawley rats 10 weeks of age weighing 190 -220g were used. Animals were allowed free access to standard rat chow and to tap water. Diabetes mellitus was induced by a single tail-vein injection of Streptozotocin (STZ 62 mg/kg of body weight, Sigma chemical company, St. Louis, MO) dissolved in 5 normal saline at day 0. The diabetic state was confirmed 72 hours later by the determination of blood glucose concentration >300 mg/dl. From day 3, all the diabetic rats received daily subcutaneous injections of 1.0-7.0 u human recombinant long-acting insulin injection (Eli Lilly & Co., Indianapolis, ID) as required to maintain the blood glucose concentration between 300-500 mg/dl. Tail blood 10 glucose levels were measured twice a week with an Accu – ChekTM Advantage meter (Roche Diagnostic Corporation, Indianapolis, IN). Body weights were taken once a week. Food and water intake were monitored daily.

Renal hypertrophy was well developed at 16 weeks prior to treatment with vehicle, BMP-7, or enalapril. At 16 weeks, animals were divided into 8 groups. 15 Group 1 were 16 weeks of DM. Group 2 were 16 weeks normal controls. Group 3 DM animals were treated with tail-vein injection of vehicle twice a week for 16 weeks. Group 4, Group 5, and Group 6 were DM animals treated with tail-vein injections of BMP-7 100, 30, and 10 µg/kg body weight respectively twice a week for 16 weeks. Group 7 were treated with Enalapril 20 mg/kg through the drinking 20 water for 16 weeks. Group 8 animals were normal control showed for 32 weeks alongside the diabetic animals. Group 1 and Group 2 animals were sacrificed at 16 weeks. The others were sacrificed at 32 weeks. All treatments began at 16 weeks and continued through 32 weeks. Glomerular filtration rate (GFR), urine albumin excretion, kidney weight, glomerular area, mesangial matrix area, interstitial 25 volume, monocyte and macrophage infiltration, thickness of glomerular basement membrane (GBM) and glomerulosclerosis were measured.

*Renal Function:* In all animals, glomerular filtration rate (GFR) was measured as the clearance of inulin. Rats were anesthetized with a Ketamine/Xylozine cocktail. A catheter was inserted into the femoral vein under a 30 dissecting microscope for infusion. Another catheter was placed into the femoral artery for collecting blood samples. Urine was collected by bladder cannulation.

After the completion of surgery, a bolus of 2 ml/kg of 3% inulin (Cypros Pharmaceutical Corp, W. Carlsbad, CA) and 0.2% p-aminohippurate (PAH) in normal saline was infused as a priming load, followed by a sustaining infusion of the same solution at the rate of 8 ml/h.kg. Urine collection was initiated for three 20-minute collection periods after an hour of equilibration. An arterial blood sample was obtained during each clearance period. Plasma and urine were analyzed for inulin.

10 *Urine Protein Excretion:* Before the clearance study, the rats were placed in individual metabolic cages for two 24 hour urine collections. Urine volume was measured and urinary protein concentration was determined with a Bio-Rad protein assay.

15 *Preparation of Kidneys:* After the clearance studies, the rats were euthanized. Both kidneys were rapidly removed and placed in ice-cold phosphate buffered saline (PBS). Kidneys were weighed and then sliced on a cold glass plate. Two 2 mm coronal sections were immersed in Histochoice and in 10% buffered formalin. Kidney sections were embedded in paraffin and cut at 3 µm and stained with hematoxylin and eosin, Gomori's Trichrome and periodic acid Schiff (PAS).

20 *Renal Morphology:* The morphometric analysis was done in a blind manner. Osteomeasure<sup>TM</sup> was used for morphometric analysis. Glomeruli were traced at x 400 magnification. Tissue sections stained by PAS were used. The measured glomerular parameters were as follows: (a) glomerular area, determined out of 60 glomeruli per group, which had vascular pole on it from randomly selected sections. (b) mesangial matrix area (defined as PAS-positive material in the mesangium), (c) ratio of the mesangial matrix area to the glomerular area, and (d) focal segmental 25 glomerulosclerosis (glomerular sclerosis was defined as global sclerosis or segments of glomerular tufts demonstrating collapsed, obliterated capillaries with sparseness of normal cellular elements). The percent of sclerotic glomeruli was determined was determined out of 150 glomeruli per animal from randomly selected sections (13).

30 Interstitial volume was determined by a point-counting technique on tissue sections stained by the Gomori's Trichrome method, and was expressed as the mean

percentage of grid points laying within the interstitial area in up to 5 fields in the cortex (1) (14).

Quantitation of monocyte/macrophage infiltration was determined by counting ED-1 antigen positive cells in tissue sections (1) (14). Kidneys were fixed 5 in Histochoice, paraffin embedded, and sectioned. Sections were dewaxed and rehydrated prior to incubation with the ED-1 antibody obtained from Harlan (Indianapolis, IN). The location of the primary antibodies was visualized using alkaline phosphatase-linked second antibody. To obtain numbers of infiltrating monocyte/macrophage in the glomeruli, 50 consecutive cross-sections of glomeruli 10 of each animal were evaluated. To obtain numbers of infiltrating monocyte/macrophage in the renal cortical tubulointerstitium, 10 consecutive non-overlapping fields were counted in each section and viewed at 400x magnification.

*Electron microscopy:* Fresh tissue was fixed in 3% (wt/vol) glutaraldehyde buffer and post-fixed in OsO<sub>4</sub>. Tissue was then dehydrated in ethanol and embedded 15 in Poly/bed 812 resin (Poly Science Inc, Warrington, PA). Thin sections were stained with uranyl acetate and lead citrate and examined using a transmission electron microscope.

*Blood pressure determinations:* The tail artery cuff method was used to follow blood pressures.

20        *In situ hybridizations:* <sup>35</sup>S-UPT labeled sense and antisense constructs were prepared as previously described (15). Briefly frozen sections (4-6 microns) were fixed in 4% formaldehyde in PBA for 20 min at room temperature. Sections were washed once in PBS at three times the normal concentration of salt. Sections were then washed three times in PBS for 5 min and in water for 2 min and once in 0.1 M 25 triethanolamine, pH 8.0 for 10 min. Additional washings for 10 min in 0.25% acetic anhydride in 0.1 M triethanolamine, pH 8.0 and twice for 2 min in 2x standard sodium citrate were performed. The sections were then dehydrated through graded ethanols followed by air drying for 5 min. This was followed by vacuum drying for 1 h at room temperature. Each slide was then hybridized for 18 h at 60°C with 10<sup>6</sup> 30 counts/min of <sup>33</sup>P radiolabeled riboprobe in 80 µl of hybridization mixture (50% formamide; 2% Denhardt's solution; 10% dextran sulfate; 0.3 M NaCl; 10 mM Tris,

pH = 8.0; 2 mM EDTA, 0.25 g/ml tRNA). The sense and antisense probes for BMP-7 were a kind gift of Kuber Sampath. The sense and antisense probes for Wnt4 were previously described (15). Slides were then treated for 20 min in 4x SSC at room temperature; 4x SSC for 5 min at room temperature; Rnase A (20 µg/ml) for 30 min 5 at 37°C in 0.5 M NaCl, 0.01 M Tris, pH = 8.0 and 1 mM EDTA; twice 2 x SSC for 5 min at room temperature; 1x SSC for 10 min at room temperature; 0.5 x SSC for 10 min at room temperature; and 0.1 x SSC for 30 min at 60°C. The sections were then dehydrated using increasing concentrations of ethanol followed by vacuum desiccation for 30 min. Slides were dipped in liquid photographic emulsion (Kodak 10 NTB2) and exposed for 1 wk at 4°C. After development, slides were counterstained with hematoxylin and eosin.

15 *Statistical Analysis:* Results were expressed as mean  $\pm$  SEM. In situ method statistical Analysis was carried out by using a one-way ANOVA, or a nonparametric ANOVA. Statistical significance was achieved if the  $p < 0.05$ . Data were analyzed using the InStat software.

### Results

General Group Comparisons: The number of animals in each group, body weight at the beginning and the study end, kidney weight, blood glucose concentration, insulin dose, urine protein excretion, GFR and the mortality data of 20 each group are summarized in Table 1. There were no significant differences in body weight gain between the different groups during the 16 weeks of treatment. Also there were no significant differences in blood glucose levels and insulin doses between the different groups during the 16 weeks of treatment. The blood glucose levels were significantly higher in the 16 week DM group than those of all the 32 weeks treatment groups, and insulin doses in the 16 week DM were significantly lower than those of all the 32 weeks treatment groups ( $p < 0.01$ ). We increased the 25 insulin doses during the study to increase blood sugar control, in order to decrease the mortality we were encountering. Specifically, the blood sugar range was decreased from 400-600 to 300-500. As can be seen from Table 1, these actions 30 were equally applied to all groups. The mortality observed in the various treatment groups was similar. The causes of death were hypoglycemia, ketoacidosis, and

anesthesia. Some animals died of unknown reasons because the biochemical data on the day of death were lacking, and autopsies were not informative, suggesting ketoacidosis or hypoglycemia as the causes.

**Table 1 Characterization of Animals in Various Groups**

Group	N	Body Weight (g)			Kidney weight (g)	Blood sugar (mg/dl)	Insulin Dose (u/wk)	Protein Ex (mg/day)	GFR (ml/min/100g bw)	Mortality	Cause-Mortality			
		Beginning	End	Weight Gain							keto-acidosis	hypoglycemia	anesthesia	unknown
NI 16	7		266.7±6.7		0.81±0.02				3.76±0.39	0.49±0.04	0/7			
NI 32	14		296.1±5.1		0.85±0.02				8.24±1.28	0.55±0.02	0/14			
DM 16	4				1.42±0.02	508.1±5.7*	8±1*	35.63±13.35	1.56±0.27	0/4				
DM 32	18	219.8±6.3	302.1±6.0	82.3±8.8	1.44±0.04	472.9±5.2	11±1	174.44±52.50	0.34±0.02	4/18	1/18	1/18	2/18	
BMP 10	9	213.4±4.1	292.5±7.0	79.2±5.5	1.19±0.03	460.0±5.5	12±1	59.46±21.84	0.59±0.07	2/9				2/9
BMP 30	9	219.1±3.6	280.2±5.9	65.6±3.6	1.19±0.03	455.8±5.7	13±1	33.02±9.11	0.62±0.09	1/9				1/9
BMP 100	10	204.8±4.9	290.3±6.6	85.5±6.9	1.10±0.03	461.6±5.5	16±1	14.27±3.50	0.70±0.08	1/10				1/10
E 100	10	216.1±4.0	294.9±4.4	78.7±5.9	1.09±0.04	459.7±5.9	14±1	33.59±7.58	0.58±0.05	3/10	1/10	1/10		1/10

\*p<0.01 vs the 32 wk groups; Other significant differences demonstrated in figures

Values are means ± SEM

5      *Characterization of the Streptozotocin Diabetic Nephropathy Model:* The effects of diabetes on kidney weight and GFR are shown in Figure 1. DM was induced at week 0. At 16 weeks of DM, kidney weight had increased 1.8 fold compared to normal ( $1.42 \pm 0.02$  versus  $0.81 \pm 0.02$  g, p<0.01), while GFR increased 3.2-fold compared to normal ( $1.56 \pm 0.27$  versus  $0.49 \pm 0.04$  ml/min/100g body wt, p<0.01). These data demonstrated that we had induced significant renal hypertrophy in this animal model. All treatments began at 16 weeks and continued through 32 weeks (or for an additional 16 weeks).

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After 16 weeks of vehicle treatment, the kidney weights were not changed ( $1.44 \pm 0.04$  g versus  $1.42 \pm 0.02$  g) (Figure 1). But the GFR of vehicle treated diabetic rats was decreased 75% to significantly lower than normal rats maintained for 32 weeks ( $0.34 \pm 0.02$  versus  $0.55 \pm 0.02$  ml/min/100gbw, p<0.05). This demonstrated that without treatment there was a change from renal hyperfiltration to renal failure in control rats during the treatment period.

On the other hand, as shown in Figure 2, BMP-7 and enalapril treated animals had significantly decreased kidney weights ( $1.10 \pm 0.03$  in the BMP-7 high dose group,  $1.09 \pm 0.04$  in enalapril treated group) compared to the diabetic vehicle-

treated rats ( $p<0.01$ ). There was a dose-dependent ordering of kidney weights in the BMP-7 treated group ( $1.19 \pm 0.03$ ,  $1.19 \pm 0.03$ ,  $1.10 \pm 0.03$ , respectively). These data demonstrated that BMP-7 and enalapril treatment partially reversed diabetic renal hypertrophy.

5       *Effects of Therapy on Kidney Hypertrophy:* While kidney weight was not affected by 16 weeks of vehicle treatment, GFR was reduced from  $1.56 \pm 0.27$  ml/min/100 g bw to  $0.34 \pm 0.02$ , which was 40% reduced from normal of  $0.55 \pm 0.02$  ( $p<0.05$ ) (Figure 1 and 3). The GFR in the BMP-7 and enalapril treatment groups was normal or greater (Figure 3). The GFR of the BMP-7 high dose group was  
10 significantly greater than the GFR of the DM group ( $0.70 \pm 0.08$  versus  $0.34 \pm 0.02$ ,  $p<0.01$ ). There was a dose-dependent ordering of GFR in the BMP-7 treated groups ( $0.59 \pm 0.07$ ,  $0.61 \pm 0.09$ , and  $0.70 \pm 0.08$ , respectively). These data indicated that the vehicle control group had developed progressive renal failure during the 16 weeks of vehicle, while the treatment groups had partial reversal of renal  
15 hypertrophy and prevention of further injury resulting in GFR significantly greater than normal at the high dose BMP-7 and dose dependently decreasing to normal with decreasing BMP-7 doses.

20       *Urine Protein Excretion:* The effects of diabetes and treatment on urine protein excretion are shown in Figure 4. Diabetic rats exhibited a pronounced increase in protein excretion rate compared with nondiabetic rats at both 16 weeks ( $35.63 \pm 13.35$  versus  $3.76 \pm 0.39$  mg/day) and 32 weeks ( $174.44 \pm 52.50$  versus  $8.24 \pm 1.28$ ,  $p<0.01$ ). This response to diabetes was reversed by BMP-7 and enalapril treatment ( $p<0.01$ , DM versus BMP10;  $p<0.001$ , DM versus BMP30, BMP100 and Enalapril). There was a significant dose-dependent ordering of protein  
25 excretion in the BMP-7 treated groups ( $59.46 \pm 21.84$ ,  $33.02 \pm 9.11$ , and  $14.27 \pm 3.50$ , respectively,  $p<0.05$  comparing low dose to high dose BMP-7).

30       *Treatment Effects on Pathology:* Compared to normal, kidneys of 16 week diabetic rats had massive glomerular hypertrophy to go along with the increase in kidney weights (Figure 5). The 16 week diabetic kidneys also had mild increases in mesangial matrix and thickening of glomerular and tubular basement membranes (Figure 5) in agreement with previous studies (16). By 32 weeks of diabetes, the

kidneys of vehicle treated rats continued to be hypertrophied, but a significant component of glomerular area was sclerotic in a focal segmental pattern (Figures 5 and 23). The sclerotic areas demonstrated increased matrix, obliteration of capillaries and sparseness of cellular elements. Electron microscopy confirmed the 5 increase in GBM thickness produced by diabetes by 16 weeks as shown in Figure 5 (data not shown). The focal-segmental nature of glomerular sclerosis in streptozotocin diabetic nephropathy is a known deviation from the human disease pathology of a diffuse global sclerosis with intermittent Kimmelstiel-Wilson nodular glomerular sclerosis lesions(17). This difference has been previously documented  
10 (13) (17).

Treatment with BMP-7 prevented the development of glomerulosclerosis along with a dose dependent reduction in the accumulation of mesangial matrix such that the intermediate and high dose BMP-7 treatments were associated with near normal glomerular histology except for incomplete resolution of glomerular 15 hypertrophy (Figures 5-7). Enalapril therapy was less effective in preventing glomerulosclerosis, and it was less effective than intermediate or high dose BMP-7 in eliminating accumulation of mesangial matrix (Figure 5).

*Morphometric Parameters:* The effect of diabetes and the various treatments on glomerular area are shown in Figure 7A. Diabetic rats had a massively larger 20 glomerular area than normal control rats ( $1.28 \pm 0.03$  versus  $0.90 \pm 0.02 \times 10^4 \mu\text{m}^2$ ,  $p < 0.001$ ) concordant with their increased kidney weights. All the treatments except vehicle partially reversed the glomerular hypertrophy ( $p < 0.001$ ). The mesangial matrix area was also increased in diabetic rats compared to all the BMP-7 and enalapril treatment groups, but the ratio of mesangial matrix area to glomerular area 25 were not different between different groups (data not shown).

As shown in Figure 7B, the cortical interstitial volume was increased from  $9.0 \pm 0.6\%$  in nondiabetic rats to  $13.1 \pm 0.7\%$  in the DM rats. High dose BMP-7 and Enalapril treatments significantly reduced the increase in interstitial volume to  $10.7 \pm 0.3\%$ ; and  $10.3 \pm 0.4\%$ , respectively,  $p < 0.01$ . DM significantly increased the 30 monocyte/macrophage infiltration both in the glomeruli ( $2.08 \pm 0.24$  vs.  $1.01 \pm 0.17$ ,  $p < 0.01$ ) and in the cortical tubulointerstitium ( $7.25 \pm 0.51$  vs.  $4.30 \pm 0.35$ ,  $p < 0.001$ )

compared to normal rats. BMP-7 and Enalapril treatment decreased the tubulointerstitial infiltration.

The effect of diabetes, BMP-7, or enalapril treatment on glomerular pathology is shown in Fig. 8. Focal segmental glomerulosclerosis (FSGS) developed 5 in diabetic rats compared with nondiabetic rats at 32 weeks ( $10.7 \pm 4.0\%$  versus  $0.7 \pm 0.2\%$ ,  $p < 0.001$ ). This degree and type of glomerulosclerosis is similar to that reported in previous studies with STZ DM (13) (17). Glomerulosclerosis was markedly reduced by BMP-7 and less so by Enalapril treatment. There was a dose-dependent ordering of the reduction in glomerulosclerosis in the BMP-7 treated 10 groups ( $4.1 \pm 1.4\%$ ,  $p < 0.05$ ;  $3.5 \pm 0.8\%$ ,  $p < 0.01$ ; and  $2.1 \pm 0.3\%$ ,  $p < 0.001$ , respectively) (Fig. 8).

*Mechanism of BMP-7 action:* A significant component of the renoprotective actions of the positive control in these studies, Enalapril, is attributed to control of hypertension in diabetes (18) (19) (20,21). Therefore, we compared blood pressures 15 between the various treatment groups in this study. As shown in Figure 9, the 16 week diabetic rats were mildly hypertensive in agreement with previous studies. Mean systolic blood pressures were  $160 + 3$  compared to  $141 + 4$  in normal control rats(17). By 20 weeks, Enalapril therapy had reversed the hypertension of the 20 diabetic rats, and Enalapril therapy maintained normotension throughout the rest of the 32 weeks. In contrast, BMP-7 therapy, had no effect on blood pressure until after 28 weeks when it began to cause reductions in hypertension until, at 32 weeks, 25 blood pressures were normalized in the BMP-7 high dose treated animals. At 32 weeks the diabetic vehicle treated rats exhibited worsening systolic hypertension (Fig. 9) and widening of their pulse pressures consistent with increasing loss of vascular pliability.

In further pursuit of the mechanism of the renoprotective actions of BMP-7 therapy, we reasoned that a critical pathogenetic action of diabetes might be induced loss of expression of a key differentiation factor/s, such as BMP-7, enabling the ill-fated injury response to be initiated. This turned out to be so as shown in Figure 10. 30 In the normal rat kidney BMP-7 is expressed in the medulla, the cortical collecting ducts and glomerular podocytes (22). By 16 weeks of diabetes, BMP-7 expression

was absent from the kidney, and this absence was maintained during therapy with vehicle (Figure 10). Treatment with either BMP-7 or Enalapril restored BMP-7 expression in its normal pattern at high levels (Figure 10). The significance of the changes in BMP-7 expression is related to the expression of another critical kidney  
5 developmental morphogen, Wnt4, during renal injury. Wnt4 is normally expressed during kidney development following BMP-7 at the time of epithelialization of the condensing metanephric mesenchyme around the tip of the ureteric bud (7,8). Wnt4 along with a reciprocal signal from the ureteric bud (BMP-7) is required for  
epithelial differentiation of the mesenchyme and tubule formation of the condensing  
10 mesenchyme leading to formation of the comma and S-shaped bodies and glomerular development. However, in tubulointerstitial injuries, Wnt4 is re-expressed but in the absence of key factors (probably the reciprocal developmental differentiation factor) it promotes interstitial fibrosis (15). As shown in Figure 11, diabetic injury is associated with Wnt4 expression throughout the kidney, and BMP-  
15 7 and Enalapril therapy do not affect Wnt4 expression. During development Wnt4 and BMP-7 interact in the development of the nephron. In the absence of BMP-7 such as in renal injuries, Wnt4 re-expression promotes TGF® induced signaling(23), and this is the likely mechanism by which Wnt4 re-expression appears to promote renal fibrogenesis (15). As shown above, BMP-7 and Enalapril therapy stimulate a  
20 successful repair reaction and reinduction of BMP-7 expression. This may indicate that the Wnt4 actions were channeled into the successful repair reaction similar to its role in nephrogenesis.

### Discussion

The data presented here demonstrate that the long-term model of  
25 streptozotocin induced diabetes is associated with the development of nephropathy and renal failure that resembles the clinical course of human diabetic nephropathy associated with Type I diabetes (24,25). By 16 weeks massive renal hypertrophy, hyperfiltration and proteinuria were established in our model. At that time, the earliest changes of glomerular mesangial matrix accumulation were detectable as  
30 previously reported (16). Over the ensuing 16 weeks during the course of the various therapies utilized here, diabetic renal injury either progressed or was partially

reversed. In the vehicle treated group, diabetic injury progressed leading to glomerular sclerosis, worsening of the proteinuria, the development of nephrotic syndrome and the development of renal insufficiency. The progression of the nephropathy to severe proteinuria and renal insufficiency was due to progression of  
5 the diabetic glomerular pathology to the level of 10% of the glomeruli being sclerotic. This severity of disease is similar to previous reports of glomerulosclerosis in STZ induced DM nephropathy (13,17,26-28).

In three groups of diabetic rats, BMP-7 was administered twice a week intravenously at (10, 30, and 100 µg/kg/bw). The effects of BMP-7 therapy were  
10 profound. BMP-7 partially restored kidney weights towards normal, restored glomerular filtration rate to normal, dose dependently eliminated proteinuria, partially reversed glomerular hypertrophy, reversed the increase in the expanded interstitial volume and prevented the development of glomerular sclerosis. The reversal of renal injury was the mechanism by which kidney weights, glomerular  
15 area, and glomerular filtration rate were reduced. All of the parameters of nephropathy that were assessed demonstrated dose ordering in their response that was small though present. Only the differences in protein excretion between the low dose and high dose groups achieved statistical significance.

The therapeutic effects of BMP-7 were further characterized by direct  
20 comparison to the known therapeutic actions of Enalapril, an angiotensin converting enzyme (ACE) inhibitor. ACE inhibition was first shown to be a renal disease therapeutic agent in STZ diabetes (18,19) (16) (20), and it is especially effective in human diabetic nephropathy (21,29) (30). Subsequently, along with AT-1 receptor blockade, it has become the main renal disease therapeutic for slowing progression  
25 of disease (29) (30). In the design of the studies reported here, a treatment of established disease was used. High dose BMP-7 was more effective than Enalapril in reversing proteinuria, maintaining GFR, decreasing mesangial matrix expansion and preventing the development of glomerular sclerosis. BMP-7 and Enalapril therapies were equally effective in reducing kidney weights, reversing glomerular hypertrophy  
30 and decreasing interstitial volume expansion. Enalapril therapy normalized blood pressure during the entire 16 week course of therapy. An important component of

the therapeutic effectiveness of Enalapril is estimated to be due to the control of systemic and glomerular hemodynamics (18-20). BMP-7 did not affect blood pressure until late in the course of the study, at a time when systolic hypertension was becoming prominent and vascular calcification was present (Davies and Hruska,  
5 data not published). We interpret these data to correlate with the actions of BMP-7 to prevent vascular calcification (31). The doses of Enalapril used here (20 mg/kg) were maximal (1,14,32). They were 10-50 fold greater than the doses used in clinical medicine. Preliminary studies demonstrated that there was no difference in the Enalapril response between 5 and 20 mg/kg. Furthermore, we have demonstrated  
10 that addition of Enalapril to the drinking water is equally effective as more arduous means of administering Enalapril such as interperitoneal injections and oral/esophageal gavage (Hruska, Wang unpublished data). Therefore, we conclude that BMP-7 therapy of streptozotocin induced diabetic nephropathy in the rat is at least equally effective to Enalapril therapy, and in the area of preventing progression  
15 to glomerular sclerosis clearly more effective in this study.

BMP-7 is a critical renal morphogen that is expressed in the normal adult kidney and lost through the influence of renal injury as shown here in diabetic nephropathy. Its therapeutic actions in renal disease appear to be related to recapitulation of its developmental actions. BMP-7 stimulates epithelial differentiation and provides metanephric mesenchymal survival signal during morphogenesis (4) (5) (6). This is similar to the actions of BMP-7 during renal tubulointerstitial injury (1). BMP-7 decreases expression of markers of injury response such as vimentin and  $\alpha$ 1(I) procollagen,  $\alpha$ 1(IV) collagen, and it increases expression of epithelial phenotype markers such as E-cadherin (1,5,9,12,22,33)  
20 (Hruska, unpublished). It prevents tubular epithelial apoptosis leading to injury prevention and disease resistance (1) (12). BMP-7 is expressed in the Wolffian duct at the time of ureteric bud development (4). It continues to be expressed in the ureteric bud and the developing collecting duct throughout development (34). In addition, BMP-7 is expressed in and required for the condensing metanephric  
25 mesenchyme at the tip of the ureteric bud and in the pretubular aggregates beginning at day 12.5(4). BMP-7 expression subsequently disappears from the comma and S-shaped bodies. In the adult kidney BMP-7 is expressed in glomerular podocytes, the  
30

thick ascending limb, distal tubule, and collecting duct (22). The strongest expression is in the collecting duct especially in the medullary segments. In the absence of BMP-7, condensation of metanephric mesenchyme is diminished and mesenchymal cells apoptose between days 12 and 14 dpc (4). This is despite 5 expression of Wnt 4, another critical tubular developmental morphogen expressed in the condensing metanephric mesenchyme at the time of formation of the pretubular aggregates (8). Wnt 4 is required and sufficient for induction of tubulogenesis from day 14dpc through development of the early nephrons (8). Wnt 4 expression is diminished and absent from the Sshaped bodies following its inductive actions.

10 From their overlapping expression patterns between day 12.5 and 14dpc, the phenotype of their knockouts, and the overlapping times of their required presence, it is clear that Wnt4 and BMP-7 interact during renal development (4) (5) (7,8). In the adult kidney, Wnt4 expression is limited to the terminal medullary collecting duct (15). However, increased Wnt 4 expression is observed throughout the 15 collecting duct in response to renal injuries while BMP-7 expression is lost, as shown here and elsewhere (1,7,15). Here we show that diabetic nephropathy causes an even more widespread expression of Wnt4 than ureteral obstruction. Wnt4 expression in response to renal injury promotes tubulointerstitial fibrosis (15), and it promotes epithelial to mesenchymal transdifferentiation (EMT) similar to TGF $\beta$  20 (15,35). This is a critical mechanism of producing interstitial myofibroblasts and promoting fibrogenesis (36). These actions of WNT4 are similar to those of TGF $\beta$  in renal injury. The mechanism of the relationship between Wnt4 and TGF $\square$  in renal injury is that Wnt 4 signaling stabilizes  $\beta$ -catenin and increases nuclear  $\beta$ -catenin levels (37,38). In the nucleus  $\beta$ -catenin binds in a transcriptional complex with 25 SMAD 4 (23). SMAD 4 in the nucleus exists as a dimer containing a regulatory SMAD. TGF- $\beta$  induces the regulatory SMAD's 2 and 3, and the SMAD 2/4 and 3/4 dimers in the transcriptional complex associated with  $\beta$ -catenin and the TCF/LEF family regulate gene transcription. The Wnt4/ TGF $\beta$  interaction leads to promotion of renal fibrosis and EMT (15) (23). Since TGF- $\beta$  is increased in response to 30 diabetic injury (26,39) (27) (28), and BMP-7 is decreased (data presented here) (40), activation of Wnt4 leads to synergism with TGF- $\beta$  (23). In the presence of BMP-7,

TGF- $\beta$  signaling is inhibited (9). BMP-7 signaling interacts with Wnt 4 expression leading to the formation of transcriptional complexes containing the BMP-7 stimulated regulatory SMAD's 1,5, and 8 as in development. Thus, the transcriptional signaling induced by TGF- $\beta$  is competitively inhibited. In addition, 5 BMP-7 stimulates the inhibitory SMAD, SMAD 6 (9), and in the proximal tubule, SMAD 7 (41), which further inhibit TGF- $\beta$  induced signaling.

The STZ induced DM rat model and type I human DM overexpress TGF $\beta$  1,2, and 3 and the TGF $\beta$  RII in renal tubular and glomerular cells(26,27,27,28,39,42). TGF $\beta$  and the receptor together constitute a major biologic 10 signal inducing a switch toward a profibrotic cellular phenotype (43). Here we demonstrate that another profibrotic cytokine, Wnt4, that synergizes with TGF $\beta$  is upregulated in STZ DM. We propose that Wnt4 upregulation may be a common response to renal injury, which, in the absence of BMP-7, stimulates a failed injury response through its interactions with TGF $\beta$ .

15 In summary, the proof of concept studies reported here demonstrate successful treatment of streptozotocin induced diabetic nephropathy in renal insufficiency with BMP-7. BMP-7 effects were equal to and in some respects more potent than a positive control, Enalapril therapy. Diabetic injury induced diffuse Wnt-4 expression representing an injury response and reexpression of a 20 developmental morphogen. In the absence of BMP-7, Wnt4 may synergize with TGF $\square$  in stimulating diabetic injury. BMP-7 therapy restored tubular epithelial phenotype, BMP-7 expression, and inhibited TGF $\beta$  actions. As a result a significant positive repair reaction was produced by BMP-7 and glomerular sclerosis was prevented as well as the development of renal insufficiency.

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#### Equivalents

25 The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes which come within

the meaning and range of equivalency of the claims are intended to be embraced therein.

SEQUENCE LISTING

**SEQ\_ID\_NO:1**

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1 5 10 15

Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser Ser  
20 25 30

Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg  
35 40 45

Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala  
50 55 60

Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn  
65 70 75 80

Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro  
85 90 95

Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile  
100 105 110

Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr  
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Arg Asn Met Val Val Arg Ala Cys Gly Cys His  
130 135

**SEQ\_ID\_NO:2**

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Ala Val Glu Glu Gly Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro  
50 55 60

Tyr Lys Ala Val Phe Ser Thr Cln Gly Pro Pro Leu Ala Ser Leu Cln  
65 \_\_\_\_\_ 70 \_\_\_\_\_ 75 \_\_\_\_\_ 80

Asp Ser His Phe Leu Thr Asp Ala Asp Met Val Met Ser Phe Val Asn  
\_\_\_\_\_ 85 \_\_\_\_\_ 90 \_\_\_\_\_ 95

Leu

~~SEQ ID NO:3~~

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala  
1 \_\_\_\_\_ 5 \_\_\_\_\_ 10 \_\_\_\_\_ 15

Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser  
\_\_\_\_\_ 20 \_\_\_\_\_ 25 \_\_\_\_\_ 30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser  
35 \_\_\_\_\_ 40 \_\_\_\_\_ 45

Gln Glu Arg Arg Glu Met Cln Arg Glu Ile Leu Ser Ile Leu Gly Leu  
50 \_\_\_\_\_ 55 \_\_\_\_\_ 60

Pro His Arg Pro Arg Pro His Leu Cln Gly Lys His Asn Ser Ala Pro  
65 \_\_\_\_\_ 70 \_\_\_\_\_ 75 \_\_\_\_\_ 80

Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly  
85 \_\_\_\_\_ 90 \_\_\_\_\_ 95

Gly Pro Gly Gly Cln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser  
100 \_\_\_\_\_ 105 \_\_\_\_\_ 110

Thr Gln Gly Pro Pro Leu Ala Ser Leu Cln Asp Ser His Phe Leu Thr  
115 \_\_\_\_\_ 120 \_\_\_\_\_ 125

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SEQ ID NO :4

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Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro  
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Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile  
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Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr  
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Arg Asn Met Val Val Arg Ala Cys Gly Cys His  
130 135

**SEQ ID NO.5**

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Arg Asn Met Val Val Lys Ala Cys Gly Cys His  
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**SEQ ID NO.6**

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Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn  
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Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro  
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Arg Asn Met Val Val Lys Ala Cys Gly Cys His  
130 135

**SEQ ID NO :7**

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Ser Asp Ser Asp Lys Ser His Arg Ser Lys Thr Asn Lys Lys Pro Ser  
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Lys Ser Asp Ala Asn Arg Gln Phe Asn Glu Val His Lys Pro Arg Thr  
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Pro Asn His Asn Lys Met Ala Val Lys Glu Gln Arg Ser His His Lys  
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Lys Ser His His His Arg Ser His Gln Pro Lys Gln Ala Ser Ala Ser

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Pro Thr Leu Val Leu Asp Arg Glu Val Ala Ser Ile Asn Val Pro Ala  
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Asn Ala Lys Ala Ile Ile Ala Glu Gln Gly Pro Ser Thr Tyr Ser Lys  
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370                   375                   380

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580 585

SEQ ID NO : 8

Met Val Trp Leu Arg Leu Trp Ala Phe Leu His Ile Leu Ala Ile Val  
1 5 10 15

Thr Leu Asp Pro Glu Leu Lys Arg Arg Glu Glu Leu Phe Leu Arg Ser  
20 25 30

Leu Gly Phe Ser Ser Lys Pro Asn Pro Val Ser Pro Pro Pro Val Pro  
35 40 45

Ser Ile Leu Trp Arg Ile Phe Asn Gln Arg Met Gly Ser Ser Ile Gln  
50 55 60

Lys Lys Lys Pro Asp Leu Cys Phe Val Glu Glu Phe Asn Val Pro Gly  
65 70 75 80

Ser Val Ile Arg Val Phe Pro Asp Gln Gly Arg Phe Ile Ile Pro Tyr  
85 90 95

Ser Asp Asp Ile His Pro Thr Gln Cys Leu Glu Lys Arg Leu Phe Phe  
100 105 110

Asn Ile Ser Ala Ile Glu Lys Glu Glu Arg Val Thr Met Gly Ser Gly  
115 120 125

Ile Glu Val Gln Pro Glu His Leu Leu Arg Lys Gly Ile Asp Leu Arg  
130 135 140

Leu Tyr Arg Thr Leu Gln Ile Thr Leu Lys Gly Met Gly Arg Ser Lys  
145 150 155 160

Thr Ser Arg Lys Leu Leu Val Ala Gln Thr Phe Arg Leu Leu His Lys  
165 170 175

Ser Leu Phe Phe Asn Leu Thr Glu Ile Cys Gln Ser Trp Gln Asp Pro  
180 185 190

Leu Lys Asn Leu Gly Leu Val Leu Glu Ile Phe Pro Lys Lys Glu Ser  
195 200 205

Ser Trp Met Ser Thr Ala Asn Asp Glu Cys Lys Asp Ile Gln Thr Phe  
210 215 220

Leu Tyr Thr Ser Leu Leu Thr Val Thr Leu Asn Pro Leu Arg Cys Lys  
225 230 235 240

Arg Pro Arg Arg Lys Arg Ser Tyr Ser Lys Leu Pro Phe Thr Ala Ser

245                    250                    255

Asn Ile Cys Lys Lys Arg His Leu Tyr Val Glu Phe Lys Asp Val Gly  
260                    265                    270

Trp Gln Asn Trp Val Ile Ala Pro Gln Gly Tyr Met Ala Asn Tyr Cys  
275                    280                    285

Tyr Gly Glu Cys Pro Tyr Pro Leu Thr Glu Ile Leu Asn Gly Ser Asn  
290                    295                    300

His Ala Ile Leu Gln Thr Leu Val His Ser Ile Glu Pro Glu Asp Ile  
305                    310                    315                    320

Pro Leu Pro Cys Cys Val Pro Thr Lys Met Ser Pro Ile Ser Met Leu  
325                    330                    335

Phe Tyr Asp Asn Asn Asp Asn Val Val Leu Arg His Tyr Glu Asn Met  
340                    345                    350

Ala Val Asp Glu Cys Gly Cys Arg  
355                    360

**SEQ ID NO: 9**

Met Arg Lys Met Gln Lys Glu Ile Leu Ser Val Leu Gly Pro Pro His  
1                        5                        10                        15

Arg Pro Arg Pro Leu His Gly Leu Gln Gln Pro Gln Pro Pro Val Leu  
20                      25                      30

Pro Pro Gln Gln Gln Gln Gln Gln Gln Gln Thr Ala Arg Glu  
35                      40                      45

Glu Pro Pro Pro Gly Arg Leu Lys Ser Ala Pro Leu Phe Met Leu Asp  
50                      55                      60

Leu Tyr Asn Ala Leu Ser Asn Asp Asp Glu Glu Asp Gly Ala Ser Glu  
65                      70                      75                      80

Gly Val Gly Gln Glu Pro Gly Ser His Gly Gly Ala Ser Ser Ser Gln  
85                      90                      95

Leu Arg Gln Pro Ser Pro Gly Ala Ala His Ser Leu Asn Arg Lys Ser

100                    105                    110

Leu Leu Ala Pro Gly Pro Gly Gly Gly Ala Ser Pro Leu Thr Ser Ala  
115                    120                    125

Gln Asp Ser Ala Phe Leu Asn Asp Ala Asp Met Val Met Ser Phe Val  
130                    135                    140

Asn Leu Val Glu Tyr Asp Lys Glu Phe Ser Pro His Gln Arg His His  
145                    150                    155                    160

Lys Glu Phe Lys Phe Asn Leu Ser Gln Ile Pro Glu Gly Glu Ala Val  
165                    170                    175

Thr Ala Ala Glu Phe Arg Val Tyr Lys Asp Cys Val Val Gly Ser Phe  
180                    185                    190

Lys Asn Gln Thr Phe Leu Ile Ser Ile Tyr Gln Val Leu Gln Glu His  
195                    200                    205

Gln His Arg Asp Ser Asp Leu Phe Leu Leu Asp Thr Arg Val Val Trp  
210                    215                    220

Ala Ser Glu Glu Gly Trp Leu Glu Phe Asp Ile Thr Ala Thr Ser Asn  
225                    230                    235                    240

Leu Trp Val Val Thr Pro Gln His Asn Met Gly Leu Gln Leu Ser Val  
245                    250                    255

Val Thr Arg Asp Gly Leu His Val Asn Pro Arg Ala Ala Gly Leu Val  
260                    265                    270

Gly Arg Asp Gly Pro Tyr Asp Lys Gln Pro Phe Met Val Ala Phe Phe  
275                    280                    285

Lys Val Ser Glu Val His Val Arg Thr Thr Arg Ser Ala Ser Ser Arg  
290                    295                    300

Arg Arg Gln Gln Ser Arg Asn Arg Ser Thr Gln Ser Gln Asp Val Ser  
305                    310                    315                    320

Arg Gly Ser Gly Ser Ser Asp Tyr Asn Gly Ser Glu Leu Lys Thr Ala  
325                    330                    335

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Cln Asp Leu Gly Trp Gln  
340 345 350

Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly  
355 360 365

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala  
370 375 380

Ile Val Cln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro Lys  
385 390 395 400

Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe  
405 410 415

Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val  
420 425 430

Arg Ala Cys Gly Cys His  
435

**SEQ\_ID\_NO:10**

gggggaettet tgaaccttgea gggagaataa ettgegeaee eaacttt geg eeggt geett 60  
tgeeeeageg gageetgett egccatetec gageeeeeaee gcccctccac teeteggeet 20  
tgeeeegaeac tgagaegets tteccagegst gaaaagagag aetgegegge eggeaeegg 180  
gagaaggagg aggeaaagaa aaggaaegga catteggtee ttgegecagg teetttgacc 240  
agagtttca eatgttggaaeg ettttcaat ggaegtgtcc eeggt gett ettagaegga 300  
etgeggtete etaaaggteg aeatggtgg cgggaaeeg etgttetteta gegtt getge 360  
tteeeeaggt ceteetggge ggegeggetg geetegttcc ggagetggge egeagggaaat 420  
tceggggc gtgttggge egeeeectat cccageccctc tgacgaggte ctgagggagt 480  
tegagtttgeg getgeteage atgttggcc tgaaaaagag aeeeaecccc ageagggaaeg 540  
eestgggtgee cccatcatg etagacctgt atgegeaggea eteaggttca ggggttca 600  
egeeeeeaga caaeggttg gagagggeag eeageegage caacaetgt egeagtttc 660  
aaeatgaaga attttggaa gaacteeag aaaegagtgg gaaaaeaace eggagattt 720  
tcttttaattt aagtttate cccaaeggagg agtttateae eteageagag etteaggttt 780  
teegagaaaca gatgeaagat getttaggaa aeaatageag tttccatca egaattaata 840

tttatgaaat cataaaaact gaaaaagcca aetegaaatt eeeeagtgaec agaettttgg 900  
aaceeagggtt ggtgaateag aatgeaagea ggtggggaaag ttttgatgte aeeeeeegetg 960  
tgtatgeggtg gaetgeacag ggacaeegea aeaatggatt cgtgggtggaa gtggcccaet 1020  
tggaggagaa aeaagggttc tecaagagae atgttaggat aageaggttc ttgeaccaaag 1080  
atgaaeacag etggteacag ataaggeat tgetagtaac ttttggcet gatggaaaag 1140  
ggeateetet eeacaaaaga gaaaaaaegt aageaaaaea caaacagegg aaaegeetta 1200  
agteecagctg taagagacac eetttgtacg tggacttcag tgaegtgggg tggaatgac 1260  
ggatttggc tccccgggg tataegeet ttaetgeea eggagaatgc eettttete 1320  
tggcgtgatea tetgaaetee aetaateatg ecattgttc gaegettggc aactetgtta 1380  
aetetaagat teetaaggea tgcgtgtcc egaeagaaat eactgetate tegatgetgt 1440  
aecttgaega gaatgaaaag gttgtattaa agaactatea ggacatggc ttggagggtt 1500  
gtgggttgc ctatcagee aaaattaaat acataaatat atatata 1547

**SEQ\_ID NO.11**

ggeagaggag gaggggaggga gggaaaggage geggageees geeeoggaaage taggtggatg 60  
tgcateega getgagggac gegageetga gaegeegtc etgcetegge tgatgtata 20  
gettgtetee eegatggat tccgtccaa getatetega geetgeageg eeacagteee 80  
eggeetegc eeaggttcac tgcacccgtt cagaggtccc caggagetgc tgcgtggc 100  
eegetactg eaggaaeta tggageeatt eegtagtgee ateeegagea aegeactget 300  
geageteee tgageetttc eageaagttt gttcaagatt ggetgtcaag aatcatggac 360  
tgttattata tgcattttt tctgtcaaga eacatgatt eetggtaacc gaatgetgt 420  
ggtegtttta ttatgecaag teetgetagg aggegegagc catgetagtt tgataactga 480  
gaegggggaaag aaaaaagtc eegagatca gggaaegeg ggaggaaegec geteaggc 540  
gaggeatgag etetgggg aettagagc gaaatttcg cagatgttc ggetgegeeg 600  
eegeeeegagc etageaaga gtgeegtc teggactac atgeggcatt tttacggc 660  
teatgtggg gaggaggagg aagageagat eeaageact ggttttgagt atetgagc 720  
eeeggeage egggeaaaea eegtgaggag ettecaaae gaagaaeate tggagaaat 780  
eeagggaaec agtggaaaact etgettttcg tttcttcc aaceteagea geateectga 840  
gaaegaggtg ateteettcg cagagtttcg gettccgg gageaggtgg aeeagggecc 900

tgattggaa agggcttcc aeegtataaa cattatgag gttatgaage eeeeageaga 960  
agtgggtgeet gggcaccta tcaeaegaet actggacaeg agaetggtee aeeaeaatgt 1020  
gaaeeggtgg gaaaactttt gatgtgagccc tgggttcctt eggtggaccc gggagaagea 1080  
geaaaaactat gggttageea ttgaggtgac tcaetttcat cagaetggaa eeeaceagg 1140  
eeageatgtc aggattagee gategttaee teaaggagc gggattttgg eeeageteeg 1200  
geeeettttg gteaeetttg geeatgtgg cggggccat geettgacccc gaegeeggag 1260  
ggeeaagegt ageectaage atcaetcaaa gggggcagg aagaagaata agaactgeeg 1320  
gegeeaeteg ctatgtgg aettttagega tgggggtgg aatgtactgg ttgtggccc 1380  
aeeaggetac eageetttt aettttagegg ggaetgeccc tttttactgg etgaccaccc 1440  
caaetcaaae aeeatgeea ttgtgeagac cttttttttt tttttttttt caatcccc 1500  
caaageetgt ttgtgtgecca etgaaactgag tgecatcttc atgetgttac tggatgagta 1560  
tgataaggcg gtaetgaaaa attateagga gatggtagta gagggatgtg ggtgeegctg 1620  
agatcagca gteettggagg atagaeagat ataaeacaaa eaaeacacaa eeaatataaa 1680  
eaaeacacaa cgttttccat eacteacaaa eaaactaaeaa agactgttcc ttatagtc 1740  
gaettttatt t 1751

**SEQ\_ID\_N\_12**

Met Ala Gly Ala Ser Arg Leu Leu Phe Leu Trp Leu Gly Cys Phe Cys  
1 5 10 15

Val Ser Leu Ala Cln Gly Glu Arg Pro Lys Pro Pro Phe Pro Glu Leu  
20 25 30

Arg Lys Ala Val Pro Gly Asp Arg Thr Ala Gly Gly Gly Pro Asp Ser  
35 40 45

Glu Leu Cln Pro Gln Asp Lys Val Ser Glu His Met Leu Arg Leu Tyr  
50 55 60

Asp Arg Tyr Ser Thr Val Gln Ala Ala Arg Thr Pro Gly Ser Leu Glu  
65 70 75 80

Gly Gly Ser Cln Pro Trp Arg Pro Arg Leu Leu Arg Glu Gly Asn Thr  
85 90 95

Val Arg Ser Phe Arg Ala Ala Ala Glu Thr Leu Glu Arg Lys Gly

100 105 110

Leu Tyr Ile Phe Asn Leu Thr Ser Leu Thr Lys Ser Glu Asn Ile Leu  
115 120 125

Ser Ala Thr Leu Tyr Phe Cys Ile Gly Glu Leu Gly Asn Ile Ser Leu  
130 135 140

Ser Cys Pro Val Ser Gly Gly Cys Ser His His Ala Gln Arg Lys His  
145 150 155 160

Ile Gln Ile Asp Leu Ser Ala Trp Thr Leu Lys Phe Ser Arg Asn Gln  
165 170 175

Ser Gln Leu Leu Gly His Leu Ser Val Asp Met Ala Lys Ser His Arg  
180 185 190

Asp Ile Met Ser Trp Leu Ser Lys Asp Ile Thr Gln Phe Leu Arg Lys  
195 200 205

Ala Lys Glu Asn Glu Glu Phe Leu Ile Gly Phe Asn Ile Thr Ser Lys  
210 215 220

Gly Arg Gln Leu Pro Lys Arg Arg Leu Pro Phe Pro Glu Pro Tyr Ile  
225 230 235 240

Leu Val Tyr Ala Asn Asp Ala Ala Ile Ser Glu Pro Glu Ser Val Val  
245 250 255

Ser Ser Leu Gln Gly His Arg Asn Phe Pro Thr Gly Thr Val Pro Lys  
260 265 270

Trp Asp Ser His Ile Arg Ala Ala Leu Ser Ile Glu Arg Arg Lys Lys  
275 280 285

Arg Ser Thr Gly Val Leu Leu Pro Leu Gln Asn Asn Glu Leu Pro Gly  
290 295 300

Ala Glu Tyr Gln Tyr Lys Lys Asp Glu Val Trp Glu Glu Arg Lys Pro  
305 310 315 320

Tyr Lys Thr Leu Gln Ala Gln Ala Pro Glu Lys Ser Lys Asn Lys Lys  
325 330 335

Lys Gln Arg Lys Gly Pro His Arg Lys Ser Gln Thr Leu Gln Phe Asp  
340 345 350

Glu Gln Thr Leu Lys Lys Ala Arg Arg Lys Gln Trp Ile Glu Pro Arg  
355 360 365

Asn Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp  
370 375 380

Ser Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ser  
385 390 395 400

Gly Ala Cys Gln Phe Pro Met Pro Lys Ser Leu Lys Pro Ser Asn His  
405 410 415

Ala Thr Ile Gln Ser Ile Val Arg Ala Val Gly Val Val Pro Gly Ile  
420 425 430

Pro Glu Pro Cys Cys Val Pro Glu Lys Met Ser Ser Leu Ser Ile Leu  
435 440 445

Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn Met  
450 455 460

Thr Val Glu Ser Cys Ala Cys Arg  
465 470

**SEQ ID NO.13**

Met Pro Pro Pro Gln Gln Gly Pro Cys Gly His His Leu Leu Leu Leu  
1 5 10 15

Leu Ala Leu Leu Leu Pro Ser Leu Pro Leu Thr Arg Ala Pro Val Pro  
20 25 30

Pro Gly Pro Ala Ala Ala Leu Leu Gln Ala Leu Gly Leu Arg Asp Glu  
35 40 45

Pro Gln Gly Ala Pro Arg Leu Arg Pro Val Pro Pro Val Met Trp Arg  
50 55 60

Leu Phe Arg Arg Arg Asp Pro Gln Glu Thr Arg Ser Gly Ser Arg Arg  
65 70 75 80

Thr Ser Pro Gly Val Thr Leu Cln Pro Cys His Val Glu Glu Leu Gly  
85 90 95

Val Ala Gly Asn Ile Val Arg His Ile Pro Asp Arg Gly Ala Pro Thr  
100 105 110

Arg Ala Ser Glu Pro Val Ser Ala Ala Gly His Cys Pro Glu Trp Thr  
115 120 125

Val Val Phe Asp Leu Ser Ala Val Glu Pro Ala Glu Arg Pro Ser Arg  
130 135 140

Ala Arg Leu Glu Leu Arg Phe Ala Ala Ala Ala Ala Ala Pro Glu  
145 150 155 160

Gly Gly Trp Glu Leu Ser Val Ala Cln Ala Gly Cln Gly Ala Gly Ala  
165 170 175

Asp Pro Gly Pro Val Leu Leu Arg Cln Leu Val Pro Ala Leu Gly Pro  
180 185 190

Pro Val Arg Ala Glu Leu Leu Gly Ala Ala Trp Ala Arg Asn Ala Ser  
195 200 205

Trp Pro Arg Ser Leu Arg Leu Ala Leu Ala Leu Arg Pro Arg Ala Pro  
210 215 220

Ala Ala Cys Ala Arg Leu Ala Glu Ala Ser Leu Leu Leu Val Thr Leu  
225 230 235 240

Asp Pro Arg Leu Cys His Pro Leu Ala Arg Pro Arg Arg Asp Ala Glu  
245 250 255

Pro Val Leu Gly Gly Pro Gly Gly Ala Cys Arg Ala Arg Arg Leu  
260 265 270

Tyr Val Ser Phe Arg Glu Val Gly Trp His Arg Trp Val Ile Ala Pro  
275 280 285

Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly Gln Cys Ala Leu Pro Val  
290 295 300

Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala Leu Asn His Ala Val Leu

305                   310                   315                   320

Arg Ala Leu Met His Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys  
325                   330                   335

Cys Val Pro Ala Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn  
340                   345                   350

Ser Asp Asn Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu  
355                   360                   365

Cys Gly Cys Arg  
370

**SEQ ID NO:14**

Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser  
1                   5                   10                   15

Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro  
20                   25                   30

Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp  
35                   40                   45

Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val  
50                   55                   60

Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His  
65                   70                   75                   80

Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu  
85                   90                   95

Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln  
100                   105                   110

Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala  
115                   120                   125

Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp  
130                   135                   140

Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu

145 150 155 160  
Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg  
165 170 175  
  
Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val  
180 185 190  
  
Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu  
195 200 205  
  
Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly  
210 215 220  
  
Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr  
225 230 235 240  
  
Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His  
245 250 255  
  
Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala  
260 265 270  
  
His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly  
275 280 285  
  
Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly  
290 295 300  
  
Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His  
305 310 315 320  
  
His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser  
325 330 335  
  
Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg  
340 345 350  
  
Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp  
355 360 365  
  
His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser  
370 375 380

Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His  
385 390 395 400

Ala Ile Val Cln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro  
405 410 415

Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr  
420 425 430

His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile  
435 440 445

Val Lys Ser Cys Gly Cys His  
450 455

**SEQ ID NO:15**

1 mhltvflkkg ivgflwscwv lvgyakggglg dnhvhssfiy rrlrnherre iqreilsilg  
61 lphrprpfsp gkqassaplf mldlynamtn eenpeesey s vraslaectr garkgypasp  
121 ngyprrriqlo rttplttqsp plaslhdtnf lndadmvmsf vnlderdkdf shqrzhykef  
181 rfdltpiqphs eavtaaeefri ykdrnnrfe netikisiyq iikeytndrda dlflldtrka  
241 qaldvgwlvf ditvtshhwv inpqnnnlglq leactgdgrs invksaglvq rqqpqskqpf  
301 mvaffkasev llrsrvraank rknqnrrnkss shqdserrmss vgdynntseqk qaekkhelyv  
361 sfredlgwqdw iiapegyyaaaf yedgeesfpl nahmnatnha ivqtvlvhlmf pdhvpkpeea  
421 ptklnaisvlyfddsonvil kkyrnmvvrs egeh

**SEQ ID NO:16**

Met Pro Gly Leu Gly Arg Arg Ala Cln Trp Leu Cys Trp Trp Trp Gly  
1 5 10 15

Leu Leu Cys Ser Cys Cys Gly Pro Pro Pro Leu Arg Pro Pro Leu Pro  
20 25 30

Ala Ala Ala Ala Ala Ala Gly Gly Gln Leu Leu Gly Asp Gly Gly  
35 40 45

Ser Pro Gly Arg Thr Glu Gln Pro Pro Pro Ser Pro Gln Ser Ser Ser  
50 55 60

Gly Phe Leu Tyr Arg Arg Leu Lys Thr Gln Glu Lys Arg Glu Met Gln  
65 70 75 80

Lys Glu Ile Leu Ser Val Leu Gly Leu Pro His Arg Pro Arg Pro Leu  
85 90 95

His Gly Leu Cln Gln Pro Gln Pro Pro Ala Leu Arg Cln Gln Glu Glu  
100 105 110

Gln Gln Gln Gln Gln Leu Pro Arg Gly Glu Pro Pro Pro Gly Arg  
115 120 125

Leu Lys Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr Asn Ala Leu Ser  
130 135 140

Ala Asp Asn Asp Glu Asp Gly Ala Ser Glu Gly Glu Arg Gln Cln Ser  
145 150 155 160

Trp Pro His Glu Ala Ala Ser Ser Ser Gln Arg Arg Gln Pro Pro Pro  
165 170 175

Gly Ala Ala His Pro Leu Asn Arg Lys Ser Leu Leu Ala Pro Gly Ser  
180 185 190

Gly Ser Gly Gly Ala Ser Pro Leu Thr Ser Ala Gln Asp Ser Ala Phe  
195 200 205

Leu Asn Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu Tyr  
210 215 220

Asp Lys Glu Phe Ser Pro Arg Gln Arg His His Lys Glu Phe Lys Phe  
225 230 235 240

Asn Leu Ser Cln Ile Pro Glu Gly Clu Val Val Thr Ala Ala Glu Phe  
245 250 255

Arg Ile Tyr Lys Asp Cys Val Met Gly Ser Phe Lys Asn Gln Thr Phe  
260 265 270

Leu Ile Ser Ile Tyr Cln Val Leu Cln Glu His Gln His Arg Asp Ser  
275 280 285

Asp Leu Phe Leu Leu Asp Thr Arg Val Val Trp Ala Ser Glu Glu Gly  
290 295 300

Trp Leu Glu Phe Asp Ile Thr Ala Thr Ser Asn Leu Trp Val Val Thr  
305 310 315 320

Pro Gln His Asn Met Gly Leu Cln Leu Ser Val Val Thr Arg Asp Gly

325 330 335

Val His Val His Pro Arg Ala Ala Gly Leu Val Gly Arg Asp Gly Pro  
340 345 350

Tyr Asp Lys Gln Pro Phe Met Val Ala Phe Phe Lys Val Ser Glu Val  
355 360 365

His Val Arg Thr Thr Arg Ser Ala Ser Ser Arg Arg Arg Cln Gln Ser  
370 375 380

Arg Asn Arg Ser Thr Cln Ser Gln Asp Val Ala Arg Val Ser Ser Ala  
385 390 395 400

Ser Asp Tyr Asn Ser Ser Glu Leu Lys Thr Ala Cys Arg Lys His Glu  
405 410 415

Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala  
420 425 430

Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly Glu Cys Ser Phe Pro  
435 440 445

Leu Asn Ala His Met Asn Ala Thr Asn His Ala Ile Val Cln Thr Leu  
450 455 460

Val His Leu Met Asn Pro Glu Tyr Val Pro Lys Pro Cys Cys Ala Pro  
465 470 475 480

Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Asn Ser Asn  
485 490 495

Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys  
500 505 510

His

**SEQ ID NO:17**

GCTGGGGGCC CGGAGCCCCGG AGCCCCCCGTA CGCCCTAGAG CCGGGGGCG ATG CAC GTG 57  
Met His Val  
1

CCC TCA CTC CGA CCT CCC CCC CCG CAC AGC TTC CTC CGG CTC TGC CCA 105  
Arg Ser Leu Arg Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala

5                    10                    15

CCC CTG TTC CTG CTC CGC TCC GCC CTG CCC GAC TTC AGC CTC GAC AAC 153  
 Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn  
 20                    25                    30                    35

GAG GTC CAC TCG AGC TTC ATC CAC CGG CGG CTC CGG ACC CAG GAG CGG 201  
 Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Cln Glu Arg  
 40                    45                    50

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CGG CAG ATG CAG CGG GAG ATC CTC TCC ATT TTG CCC TTG CCC CAC CGG 249  
 Arg Glu Met Cln Arg Glu Ile lieu Ser Ile Leu Gly Leu Pro His Arg

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55                    60                    65

CCC CCC CGG CAC CTC CAG CGC AAG CAC AAC TCG CCA CCC ATG TTC ATG 297  
 Pro Arg Pro His Leu Cln Gly Lys His Asn Ser Ala Pro Met Phe Met  
 70                    75                    80

CTG GAC CTG TAC AAC CGC ATG CGC CTG GAG CGG CGG CCC CCC CGC 345  
 Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Pro Gly  
 85                    90                    95

GGC CAG GGC TTC TCC TAC CCC TAC AAG GGC CTC TTC AGT ACC CAG CGC 393  
 Gly Cln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Cln Gly  
 100                    105                    110                    115

CCC CCT CTG CCC AGC CTG CAA GAT ACC CAT TTC CTC ACC GAC CCC GAC 441  
 Pro Pro Leu Ala Ser Leu Cln Asp Ser His Phe Leu Thr Asp Ala Asp  
 120                    125                    130

ATG GTC ATG AGC TTC GTC AAC CTC CTG GAA CAT GAC AAC GAA TTC TTC 489  
 Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe  
 135                    140                    145

CAC CCA CGG TAC CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAC ATC 537  
 His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile  
 150                    155                    160

CCA GAA CGG CAA CCT GTC ACC (,CA GCC CAA TTC CGG ATC TAC AAG GAC  
 585  
 Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp  
 165                    170                    175

TAC ATC CGG GAA CGG TTC CAC AAT GAC ACC TTC CGG ATC ACC GTT TAT 633  
 Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr  
 180                    185                    190                    195

CAG CTG CTC CAG GAC CAC TTC CGG ACC GAA TCG CAT CTC TTC CTG CTC 681  
 Gln Val Leu Cln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu  
 200                    205                    210

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GAC ACC CGT ACC CTC TGG CCC TCG GAG GAG CGG TCG CTC CTC CTC TTT GAC 729  
 Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp  
 215                    220                    225

ATC ACA GCC ACC ACC AAC CAC TGG CTC CTC AAT CCC CGG CAC AAC CTG 777  
 Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu  
 230 235 240

CGC CTG CAG CTC TCG GTG GAG ACC CTC GAT CCC CGG CAG AGC ATC AAC CCC 825  
 Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro  
 245 250 255

AAG TTG CGC CCC CTG ATT CGG CGG CAC CCC CAG AAC AAC AAC CAG CCC 873  
 Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro  
 260 265 270

TTC ATC CTG CCT TTC TTC AAC GCC ACC GAC CTC CAC TTC CCC AGC ATC 921  
 Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile  
 280 285 290

CGC TCC ACC CGG AGC AAA CAG CGC ACC CAG AAC CCC TCC AAG ACC CCC 969  
 Arg Ser Thr Gly Ser Lys Cln Arg Ser Gln Asn Arg Ser Lys Thr Pro  
 295 300 305

AAG AAC CAG CAA CCC CTG CGG ATG CCC AAC CTG CCA CAG AAC ACC ACC 1017  
 Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser  
 310 315 320

AGC CAC CAG AGC CAC CCC TGT AAG AAG CAC CAG CTC TAT GTC ACC TTC 1065  
 Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe  
 325 330 335

CGA GAC CTG CGC TGG CAC GAC TGG ATC ATC GCG CCT GAA CCC TAC CCC 1113  
 Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala  
 340 345 350 355

GCC TAC TAC TGT GAC CGG GAG TGT CCC TTC CCT CTC AAC TCC TAC ATG 1161  
 Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met  
 360 365 370

AAC CCC ACC AAC CAC CCC ATC CTC CAG ACC CTC GTC CAC TTC ATC AAC 1209  
 Asn Ala Thr Asn His Ala Ile Val Cln Thr Leu Val His Phe Ile Asn  
 375 380 385

CGC CAA ACC CTG CCC AAC CCC TGC TCT CGG CCC ACC CAG CTC AAT CCC 1257  
 Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala  
 390 395 400

ATC TCC CTC CTC TAC TTC CAT GAC ACC TCC AAC GTC ATC CTC AAG AAA 1305  
 Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys  
 405 410 415

TAC AGA AAC ATG GTC CTC CGG CCC TGT CGC TGC CAC TAGCTCCTCC 1351  
 Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His  
 420 425 430

GAGAATTGAG ACCCTTTGGG GCCAAGTTTT TCTGGATGCT CCATTGCTCG CCTTGGCCAG 1411

GAACCACGAG ACCAACTGCC TTTTGTGAGA CCTTCCCCCTC CCTATCCCCA ACTTTAAAGC 1471

TCTGAGACTA TTACCAAACA TGACCACCAT ATGGCTTTG ATCACTTTT CACTGGCACC 1531  
 ATCCAATCAA CAAGATCCTA CAACCTCTCC AGCCAAAACC TAGCACCAAA AAAAACAAAC 1591  
 CCTATAAAGAA AAATCCCCCG CCCACGTCAT TGCCTGGAA CTCTCACCCCA TCCACGGACT 1651  
 CGTTTCCACA GCTAATTATG AGCCCCTACC ACCCAGGGCA CCCAGCCCTG CGAGGAAGGG 1711  
 CGGCTGCCAA CGGGTGGGCA CATTGGTGTC TGTGGAAAG GAAAATTGAC CGGAAACTTC 1771  
 CTGTAATAAA TGTCACAATA AAACGAATGA ATCAAAAAAA AAAAAAAA A 1822

**SEQ ID NO. 18**

CTGCCACCAAC TCACCTCCCC TCGTGGACCC CTCCCCCTCCC CCCTCCCCCTG CCACCTGGGG 60  
 CCCCCCCCCC CCCCTCCCCC CGATCCCCCG TAGAGCCCCC CGC ATG CAC GTC CGC 115  
 Met His Val Arg  
 1  
 TCG CTC CGC CCT CGC CCA CAC ACC TTC GTC CGG CTC TGG CGG CCT 163  
 Ser Leu Arg Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro  
 5 10 15 20  
 CTC TTC TTG CTC CGC TCC CGC CTC CCC GAT TTC ACC CTC GAC AAC GAC 211  
 Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu  
 25 30 35  
 CTG CAC TCC ACC TTC ATC CAC CGG CGG CTC CGG ACC CAC GAG CGG CGG 259  
 Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Cln Glu Arg Arg  
 40 45 50  
 GAC ATC CAG CGG GAG ATC CTC TCC ATC TTA CGG TTC CCC CAT CGG CGG 307  
 Glu Met Cln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro  
 55 60 65  
 CCC CGG CAC CTC CAG CGA AAC GAT AAT TCG CGG CCC ATG TTC ATG TTC 355  
 Arg Pro His Leu Cln Gly Lys His Asn Ser Ala Pro Met Phe Met Leu  
 70 75 80  
 GAC CTG TAC AAC CGC ATG CGG CTC CAG GAG ACC CGG CGG GAC GGA GAG 403  
 Asp Leu Tyr Asn Ala Met Ala Val Glu Ser Gly Pro Asp Gly Cln  
 85 90 95 100  
 CCC TTC TCC TAC CCC TAC AAG CGC GTC TTC AGT ACC CAG CGG CCC CCT 451  
 Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Cln Gly Pro Pro  
 105 110 115  
 TTA GCC ACC CTC CAG GAC ACC CAT TTC CTC ACT GAC CGG GAC ATG CTC 499  
 Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp Met Val  
 120 125 130  
 ATG AGC TTC GTC AAC CTA GTC GAA CAT GAC AAA CAA TTC TTC CAC CCT 547  
 Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe His Pro  
 135 140 145

CGA TAC CAC CAT CGG GAC TTC CGG TTT CAT CTT TCC AAC ATC CCC GAC 595  
 Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile Pro Glu  
 150 155 160

CCC GAA CGG CTG ACC GCA CGG GAA TTC ACC ATC TAT AAC GAC TAC ATC 643  
 Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp Tyr Ile  
 165 170 175 180

CGG GAG CGA TTT GAC AAC GAG ACC TTC CAG ATC ACA GTC TAT CAG CTC 691  
 Arg Glu Arg Phe Asp Asn Glu Thr Phe Cln Ile Thr Val Tyr Cln Val  
 185 190 195

CTC GAG GAC GAC TCA CGC AGG GAG TCG CAC CTC TTC TTG CTG CAC AGC 739  
 Leu Cln Glu His Ser Gly Arg Glu Ser Asp Leu Phe Leu Leu Asp Ser  
 200 205 210

CCC ACC ATC TGG CCT TCT GAG GAC CGG TGG TTG CTG TTT GAT ATC ACA 787  
 Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp Ile Thr  
 215 220 225

CGC ACC ACC AAC CAC TGG GTG GTC AAC CCT CGG GAC AAC CTG CGG TTA 835  
 Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu Gly Leu  
 230 235 240

CAG CTC TCT GTG GAG ACC CTG CAT CGG CAG AGC ATC AAC CCC AAG TTG 883  
 Cln Leu Ser Val Glu Thr Leu Asp Gly Cln Ser Ile Asn Pro Lys Leu  
 245 250 255 260

CGA CGG CTG ATT CGA CGG CAT CGA CCC CAG AAC AAC CAA CCC TTC ATG 931  
 Ala Gly Leu Ile Gly Arg His Gly Pro Cln Asn Lys Cln Pro Phe Met  
 265 270 275

CTG CCC TTC TTC AAC CGG ACC GAA GTC CAT CTC CGT ACT ATC CGG TCC 979  
 Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg Ser Ile Arg Ser  
 280 285 290

ACC CGG CGG AAC CAC CGG ACC CAC AAT CGG TCC AAC ACC CCA AAC AAC 1027  
 Thr Gly Gly Lys Cln Arg Ser Cln Asn Arg Ser Lys Thr Pro Lys Asn  
 295 300 305

CAA GAG CGG CTG AGC ATC CGG AGT CTC GCA GAA AAC ACC ACC ACT GAC 1075  
 Cln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser Asp  
 310 315 320

CAG AGC CAG CGG TGC AAC AAA CAT GAC CTG TAC GTC ACC TTC CGA GAC 1123  
 Cln Arg Cln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp  
 325 330 335 340

CTT CGG TGG CAG GAC TGG ATC ATT CGA CCT GAA CGG TAT CCT CCC TAC 1171  
 Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr  
 345 350 355

TAC TGT GAG CGA GAG TGC CGG TTC CCT CTC AAC TCC TAC ATC AAC CGC 1219  
 Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala  
 360 365 370

ACC AAC CAC CGG ATC CTC CAG ACA CTC CTT CAC TTC ATC AAC CCA GAC 1267  
 Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Asp

375                    360                    385

ACA CTA CCC AAG CCC TGC TCT GCG CCC ACC CAG CTC AAC CCC ATC TCT                    1315  
 Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Cln Leu Asn Ala Ile Ser  
 390                    395                    400

GTC CTC TAC TTC GAC GAC ACC TCT AAT GTC ATC CTC AAG AAC TAC AGA                    1363  
 Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg  
 405                    410                    415                    420

AAC ATG GTC GTC CGG CCC TGT GGC TGC CAC TAGCTCTTCC TGAGACCCCTG                    1413  
 Asn Met Val Val Arg Ala Cys Gly Cys His  
 425                    430

ACCTTTGGGG CCCCACACCT TTCCAAATCT TCGATCTCTC ACCATCTAAC TCTCTCACTG                    1473  
 CCCACCTTGG CGAGGAGAAC AGACCAACCT CTCCCTGACCC TTCCCTCACC TCCCAACCCG                    1533  
 AACCATGTAA CGGTTCCAGA AACCTGAGCG TGCAGCAGCT CATGAGGGCC CTTTCCTTCT                    1593  
 GGCAACGTGAC CGACAAGATC CTACCGAGCTA CCACAGCAGA CGCCTAACAGC GAGGAAAAAT                    1653  
 GTCTGCCAGC AAAGTGTCCA GTGTCCACAT GGCCTCTGGC GCTCTGACTC TTTGAGGACT                    1713  
 AATCCCAAGC CTCCTTCAGC TGCACCCAGAA GGAACGGCTT AGCCAGGCTG GGCCTGGCC                    1773  
 TCTCTGTTCA AGCGAAACCA ACCACAAGCC ACTGTAATGA TATGTCACAA TAAAACCCAT                    1833  
 GAATGAAAAA AAAAAAAAAA AAAAAAAAAA AAAAGAATTTC                    1873

**SEQ ID NO. 19**

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala  
 1                    5                    10                    15

Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser  
 20                    25                    30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser  
 35                    40                    45

Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu  
 50                    55                    60

Pro His Arg Pro Arg Pro His Leu Cln Gly Lys His Asn Ser Ala Pro  
 65                    70                    75                    80

Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly  
 85                    90                    95

Pro Asp Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr  
 100                    105                    110

Gln Gly Pro Pro Leu Ala Ser Leu Cln Asp Ser His Phe Leu Thr Asp  
 115                    120                    125

Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu  
 130 135 140

Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser  
 145 150 155 160

Lys Ile Pro Glu Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr  
 165 170 175

Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr  
 180 185 190

Val Tyr Gln Val Lau Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe  
 195 200 205

Leu Leu Asp Ser Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val  
 210 215 220

Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His  
 225 230 235 240

Asn Leu Gly Lau Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile  
 245 250 255

Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys  
 260 265 270

Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg  
 275 280 285

Ser Ile Arg Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys  
 290 295 300

Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn  
 305 310 315 320

Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val  
 325 330 335

Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly  
 340 345 350

Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser  
 355 360 365

Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe  
 370 375 380

Ile Asn Pro Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu  
 385 390 395 400

Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu  
 405 410 415

Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His  
 420 425 430

**SEQ\_ID\_NO:20**

GGGGGGGGCGCA GAGCAGGAGT CCCTGGAGGA CCTGTGCTTC GACCCAGGACC TGGCACGGCA 60  
 CCCCTGGAGG CCTCCCTATG ACTGGGGGAC ACCGGGGCAGG AGGGGGCTGGA CCAACAGCTC 120  
 CCACACGGCA CCAAGGGGTG CCTGGAGGAC CTGGGGCATC CCCCCCTGGGG TGCTGGGACC 180  
 GGGGGCACAG CGGACTGGC CGGTACGGGG CGCACAGAGG CATTGGGGGA GAGTCCCAGT 240  
 CGGGAGAGTA GCGGGGGCT CGAGGGCTG CGGTCCCCGGT CCTCTCCCTC CAGGAGGGAG 300  
 GACAGGTGTC CGGGGGGGGC CCTCCAGGCA CGGGGGCTGA CGGGGGCTCC CGGGGGCTCC 360  
 CGGGGGGGCC CGGGGGGGCC CGGGGGGGCA CGGGGGCTCC CTTGGGGCTCC CGGGGGCTCC 420  
 AGGGGGCTGGG TCGGGGGGGG ACGGGATGCC CGGGGGCTGA CGGGGGCAGG TGAGGGGGCC 480  
 CGGGGGCTGCC ATG ACC GCG CTC CCC CGC CTC TGG CTC CTC CCC CTG 528  
 Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu  
 1 5 10

CCC CTA TCC CGC CTG CGC CGC CGC CCC CGC CGC CGA CCC CCC CCC CCC 576  
 Ala Leu Cys Ala Leu Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro  
 15 20 25

CGC TGT CCC CAG CGA CGT CTG CGC CGC CGC GAG CGC CGG CAC CTC CAG 624  
 Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln  
 30 35 40 45

CGC GAG ATC CTC CGC CTG CTC CGC CGT CCT CGG CGG CCC CGG CCC CCC CCC 672  
 Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg  
 50 55 60

CGC CCA CCC CCC CCC TCC CGC CTC CCC CGC TCC CGG CCC CTC TTC ATG 720  
 Ala Pro Pro Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met  
 65 70 75

CTG GAC CTG TAC CAC CGC ATG CGC CGC GAC GAC GAC GAC GAC CCC CCC 768  
 Leu Asp Leu Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala  
 80 85 90

CCC CGG GAG CGG CCC CTC CGC CCC CGC GAC CTG CTC ATG AGC TTC GTT 816  
 Pro Ala Glu Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val  
 95 100 105

AAC ATG CTG GAC CGA GAC CGT CGC CTG CGC CAC CAC GAG CCC CAT TGG 864  
 Asn Met Val Glu Arg Asp Arg Ala Leu Gly His Cln Glu Pro His Trp  
 110 115 120 125

AAC GAG TTC CGC TTT GAC CTG ACC CAG ATC CGG CCT CGG GAC CCC GTC 912  
 Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val  
 130 135 140

ACA GGT CGG GAG TTC CGG ATT TAC AAG CTG CCC ACC ATC CAC CTC CTC 960  
 Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu  
 145 150 155

AAC AGC ACC CTC CAC GTC ACC ATC TIC CAG GTC CTC CAC GAC CAC TCC 1008  
 Asn Arg Thr Leu His Val Ser Met Phe Cln Val Val Cln Glu Gln Ser  
 160 165 170  
  
 AAC AGC GAC TCT GAC TTG TTC TTT TTG CAT CTT CAC ACC CTC CGA CCT 1056  
 Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Cln Thr Leu Arg Ala  
 175 180 185  
  
 CGA GAC GAC CCC TGG CTC GTG CTG GAT GTC ACA GCA CCC ACT GAC TGC 1104  
 Gly Asp Glu Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys  
 190 195 200 205  
  
 TCG TTG CTG AAC CGT CAC AAG GAC CTG GGA CTC CCC CTC TAT CTG CAC 1152  
 Trp Leu Leu Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu  
 210 215 220  
  
 ACT CAC CAC CCC CAC ACC GTC GAT CCT CCC CTC CCC CCC CTC CTC CGT 1200  
 Thr Glu Asp Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly  
 225 230 235  
  
 CAA CGG CCC CCA CGC TCC CAA CAG CCT TTC GTC GTC ACT TTC TTC AGG 1248  
 Gln Arg Ala Pro Arg Ser Gln Cln Pro Phe Val Val Thr Phe Phe Arg  
 240 245 250  
  
 CGC AGT CGG ACT CCC ATC CGC ACC CCT CCG GCA GTC AGG CCA CTG AGG 1296  
 Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg  
 255 260 265  
  
 AGG AGG CAC CGG AAG AAA AGC AAC GAC CTG CCC CAG CCC AAC CGA CTC 1344  
 Arg Arg Cln Pro Lys Ser Asn Glu Leu Pro Cln Ala Asn Arg Leu  
 270 275 280 285  
  
 CCA CGG ATC TTT GAT GAC GTC CAC GGC TCC CAC CCC CGG CAG GTC TGC 1392  
 Pro Gly Ile Phe Asp Val His Gly Ser His Gly Arg Cln Val Cys  
 290 295 300  
  
 CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG GAC CTC CGC TGC CTG CAC 1440  
 Arg Arg His Glu Leu Tyr Val Ser Phe Cln Asp Leu Gly Trp Leu Asp  
 305 310 315  
  
 TGG GTC ATC GCT CCC CAA GGC TAC TCC CCC TAT TAC TGT GAG GGG CAC 1488  
 Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu  
 320 325 330  
  
 TGC TCC TTC CCA CTC CAC TCC TCC ATC AAT GCC ACC AAC CAC CCC ATC 1536  
 Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile  
 335 340 345  
  
 CTC CAG TCC CTC GTC CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG CGG 1584  
 Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala  
 350 355 360 365  
  
 TGC TGT CGA CCC ACC AAG CTC AGC CCC ACC TCT GTC CTC TAC TAT GAC 1632  
 Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp  
 370 375 380  
  
 AGC AGC AAC AAC GTC ATC CTG CCC AAA CAC CCC AAC ATC GTC CTC AAC 1680  
 Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val Lys  
 385 390 395

CCC TCC CGC TGC CAC T CAGTCAGCCCC GCCCCAGCCCT ACTGCCAC 1723  
 Ala Cys Gly Cys His  
 \_\_\_\_\_  
 400

SEQ ID NO. 21

Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys 15  
 1 5 10 15  
 Ala Leu Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro Gly Cys Pro 30  
 20 25 30  
 Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln Arg Glu Ile 45  
 35 40 45  
 Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Pro Pro 60  
 50 55 60  
 Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu 80  
 65 70 75 80  
 Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala Pro Ala Glu 95  
 85 90 95  
 Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val 110  
 100 105 110  
 Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp Lys Glu Phe 125  
 115 120 125  
 Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala 140  
 130 135 140  
 Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu Asn Arg Thr 160  
 145 150 155 160  
 Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser Asn Arg Glu 175  
 165 170 175  
 Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp Glu 190  
 180 185 190  
 Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu 205  
 195 200 205  
 Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp 220  
 210 215 220  
 Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala 240  
 225 230 235 240  
 Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro 255  
 245 250 255  
 Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln 270  
 260 265 270

Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile  
 \_\_\_\_\_  
 275 280 285

Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His  
 \_\_\_\_\_  
 290 295 300

Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile  
 \_\_\_\_\_  
 305 310 315 320

Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe  
 \_\_\_\_\_  
 325 330 335

Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser  
 \_\_\_\_\_  
 340 345 350

Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala  
 \_\_\_\_\_  
 355 360 365

Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn  
 \_\_\_\_\_  
 370 375 380

Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val Lys Ala Cys Gly  
 \_\_\_\_\_  
 385 390 395 400

Cys His

**SEQ\_ID NO+22**

GCCAGGGACA CGTGGGGGCT CTGGTCCTCC CCCTCTGGCG TCAGGGAGCC CCCACCGAGCT 60

ACCACTGGAT CGGGGGGGGC TGAAAGTCCC AG ATC GCT ATG CGT CCC CGC CCA 113  
 \_\_\_\_\_  
 Met Ala Met Arg Pro Gly Pro  
 1 5

CTC TCG CTA TTG CCC CTT CCT CTC TGC CCC CTG CGA CGC CGC CAC GGT 161  
 Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly Gly His Gly  
 \_\_\_\_\_  
 10 15 20

CCC CGT CCC CGG CAC ACC TGT CCC CAG CGT CGG CTC CGA CCC CCC GAG 209  
 Pro Arg Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu  
 \_\_\_\_\_  
 25 30 35

CCC CGG GAC ATG CAC CGT GAA ATC CTG CGG CTG CTC CGG CTA CCC CGA 257  
 Arg Arg Asp Met Gln Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly  
 \_\_\_\_\_  
 40 45 50 55

CCC CCC CGA CCC CGT CCA CAA CCC CGG CCT CCC CGG CAG CCA CCC TCC 305  
 Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala Arg Gln Pro Ala Ser  
 \_\_\_\_\_  
 60 65 70

CCC CCC CTC TTC ATC TTG CAC CTA TAC CAC GCC ATC ACC GAT GAC GAC 353  
 Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr Asp Asp Asp  
 \_\_\_\_\_  
 75 80 85

GAC CGG CGG CCA CCA CAG CCT GAC TTA CGC CGT CCC GAC CTG GTC ATG 401  
 Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp Leu Val Met  
 \_\_\_\_\_  
 90 95 100

ACC TTC GTC AAC ATG GTC CAA CCC GAC CCT ACC CTC GGC TAC CAC GAG 449  
 Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly Tyr Gln Glu  
 105 110 115

CCA CAC TGG AAG GAA TTC CAC TTT GAC CTA ACC CAC ATC CCT GCT CGG 497  
 Pro His Trp Lys Glu Phe His Phe Asp Leu Thr Gln Ile Pro Ala Gly  
 120 125 130 135

GAG CCT GTC ACA CCT GCT GAG TTC CGG ATC TAC AAA GAA CCC ACC ACC 545  
 Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Glu Pro Ser Thr  
 140 145 150

CAC CGG CTC AAC ACA ACC CTC CAC ATC ACC ATG TTC GAA CTC GTC CAA 593  
 His Pro Leu Asn Thr Thr Leu His Ile Ser Met Phe Glu Val Val Glu  
 155 160 165

GAG CAC TCC AAC ACC GAG TCT GAC TTG TTC TTT TTC GAT CTT CAG ACC 641  
 Glu His Ser Asn Arg Glu Ser Asp Leu Phe Leu Asp Leu Gln Thr  
 170 175 180

CTC CGA TCT GGG GAC GAG CGC TGG CTG CTC CTG GAC ATC ACA GCA GGC 689  
 Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu Asp Ile Thr Ala Ala  
 185 190 195

AGT GAC CGA TGG CTC CTC AAC CAT CAC AAC GAC CTC CGA CTC CCC CTC 737  
 Ser Asp Arg Trp Leu Leu Asn His His Lys Asp Leu Gly Leu Arg Leu  
 200 205 210 215

TAT GTG GAA ACC CCC GAT CGG CAC AGC ATC GAT CCT GGC CTG CCT GGT 785  
 Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp Pro Gly Leu Ala Gly  
 220 225 230

CTG CTT CGA CGA CAA GCA CGA CGC TCC AGA CAG CCT TTC ATG GTA ACC 833  
 Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln Pro Phe Met Val Thr  
 235 240 245

TTC TTC AGG CCC ACC CAC ACT CCT CTG CGG CCC CCT CGG CCA CCC AGA 881  
 Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala Pro Arg Ala Ala Arg  
 250 255 260

CCA CTG AAG AGG ACC CAG CCA AAG AAA ACC AAC GAG CTT CGG CAC CCC 929  
 Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu Pro His Pro  
 265 270 275

AAC AAA CTC CGA CGG ATC TTT GAT GAT CGC CAC CCT TCC CCC CGG AGA 977  
 Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser Arg Gly Arg  
 280 285 290 295

GAG CTT TGC CGG AGG CAT CAG CTC TAC CTC AGC TTC CGT CAC CTT GGC 1025  
 Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly  
 300 305 310

TGG CTG GAC TGG CTC ATC CCC CGG CCC TAC TCT GGC TAT TAC TGT 1073  
 Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys  
 315 320 325

GAG CCC GAG TGT CCT TTC CCA CTC GAC TCC TGT ATG AAC CCC ACC AAC 1121  
 Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn

330                    335                    340

CAT CCC ATC TTG CAG TCT CTG GTC CAC CTC ATG AAG CCA CAT GTT GTC                    1169  
His Ala Ile Leu Cln Ser Leu Val His Leu Met Lys Pro Asp Val Val  
345                    350                    355

CCC AAG CCA TGC TGT GCA CCC ACC AAA CTG. AGT CCC ACC TCT GTC CTG                    1217  
Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu  
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TAC TAT GAC ACC ACC AAC AAT GTC ATC CTG CGT AAA GAC CGT AAC ATG                    1265  
Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met  
380                    385                    390

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Val Val Lys Ala Cys Gly Cys His  
395

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1926

**SEQ ID NO :23**

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Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu  
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Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala  
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Ala Ala Arg Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr  
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His Ala Met Thr Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu  
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Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp  
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Arg Thr Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp  
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Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg  
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Ile Tyr Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile  
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Ser Met Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu  
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Phe Phe Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu  
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Val Leu Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His  
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Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser  
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Met Asp Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser  
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Arg Ala Pro Arg Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys  
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Thr Asn Glu Leu Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp  
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Gly His Gly Ser Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr  
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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala Xaa Xaa Xaa Xaa Xaa Xaa  
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Xaa Cys Cys Xaa Pro  
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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
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Xaa  
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Xaa Cys Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa  
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Xaa Xaa Xaa Xaa Xaa Xaa Val Xaa Leu Xaa Xaa Xaa Xaa Met Xaa Val  
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All cited references (including scientific publications, abstracts, etc.),  
patents, patent application publications are hereby incorporated herein by reference.

**We claim:**

1. A method of treating or preventing chronic renal failure in a mammal, comprising conjointly administering to said mammal an OP/BMP morphogen and an Angiotensin-Converting Enzyme Inhibitor (ACEI).
2. A method of treating or preventing chronic renal failure in a mammal, comprising conjointly administering to said mammal an OP/BMP morphogen and an Angiotensin II Receptor Antagonist (AIIRA).
3. A method of treating or preventing chronic renal failure in a mammal, comprising conjointly administering to said mammal an inducer of endogenous OP/BMP morphogen expression and an Angiotensin-Converting Enzyme Inhibitor (ACEI).
4. A method of treating or preventing chronic renal failure in a mammal, comprising conjointly administering to said mammal an inducer of endogenous OP/BMP morphogen expression and an Angiotensin II Receptor Antagonist (AIIRA).
5. A method of treating or preventing chronic renal failure in a mammal, comprising conjointly administering to said mammal an agonist of an OP/BMP morphogen receptor and an Angiotensin-Converting Enzyme Inhibitor (ACEI).
6. A method of treating or preventing chronic renal failure in a mammal, comprising conjointly administering to said mammal an agonist of an OP/BMP morphogen receptor and an Angiotensin II Receptor Antagonist (AIIRA).
7. A method of treating or preventing chronic renal failure in a mammal, comprising introducing into the kidney of said mammal a therapeutically effective amount of renal mesenchymal progenitor cells pre-treated

conjointly with an ACEI and an agent that increases the abundance of an OP/BMP morphogen.

8. A method of treating or preventing chronic renal failure in a mammal, comprising introducing into the kidney of said mammal a therapeutically effective amount of renal mesenchymal progenitor cells pre-treated conjointly with an AIIRA and an agent that increases the abundance of an OP/BMP morphogen.
9. The method of claim 7 or 8, wherein the agent is an OP/BMP morphogen.
10. The method of claim 7 or 8, wherein the agent is an inducer of an OP/BMP morphogen.
11. The method of claim 7 or 8, wherein the agent is an agonist of an OP/BMP morphogen receptor.
12. A method for delaying the need for, or reducing the frequency of, chronic dialysis treatments, comprising conjointly administering to a mammal an OP/BMP morphogen and an ACEI.
13. A method for delaying the need for, or reducing the frequency of, chronic dialysis treatments, comprising conjointly administering to a mammal an OP/BMP morphogen and an AIIRA.
14. A method for delaying the need for, or reducing the frequency of, chronic dialysis treatments, comprising conjointly administering to said mammal an inducer of endogenous OP/BMP morphogen expression and an ACEI.
15. A method for delaying the need for, or reducing the frequency of, chronic dialysis treatments, comprising conjointly administering to said mammal an inducer of endogenous OP/BMP morphogen expression and an AIIRA.
16. A method for delaying the need for, or reducing the frequency of, chronic dialysis treatments, comprising conjointly administering to said mammal an agonist of an OP/BMP morphogen receptor and an ACEI.

17. A method for delaying the need for, or reducing the frequency of, chronic dialysis treatments, comprising conjointly administering to said mammal an agonist of an OP/BMP morphogen receptor and an AIIRA.
18. A method as in any one of claims 1-17, wherein said mammal is afflicted with a condition selected from: chronic renal failure (CRF), end-stage renal disease (ESRD), chronic diabetic nephropathy, diabetic glomerulopathy, diabetic renal hypertrophy, hypertensive nephrosclerosis, hypertensive glomerulosclerosis, chronic glomerulonephritis, hereditary nephritis, or renal dysplasia.
19. A method as in any one of claims 1-17, wherein examination of a renal biopsy of said mammal indicates that said mammal is afflicted with a condition selected from: glomerular hypertrophy, tubular hypertrophy, glomerulosclerosis, or tubulo interstitial sclerosis.
20. A method as in any one of claims 1-17, wherein examination of said mammal indicates renal fibrosis.
21. The method of claim 20, wherein said examination is an ultrasound, NMR or CAT scan of said mammal.
22. A method as in any one of claims 1-17, wherein said mammal possesses a number of functional nephron units which is less than about 40% of a number of functional nephron units present in a mammal having intact healthy kidneys.
23. The method of claim 22, wherein said mammal possesses a number of functional nephron units which is less than about 20% of a number of functional nephron units present in a mammal having intact healthy kidneys.
24. The method of any one of claims 1-17, wherein said mammal is a kidney transplant recipient.

25. The method of any one of claims 1-17, wherein said mammal possesses only one kidney.
26. The method of any one of claims 1-17, wherein examination of a urinary sediment of said mammal indicates a presence of broad casts.
27. The method of any one of claims 1-17, wherein said mammal has a GFR which is chronically less than about 40% of a GFR<sub>exp</sub> for said mammal.
28. The method of claim 27, wherein said mammal has a GFR which is chronically less than about 20% of a GFR<sub>exp</sub> for said mammal.
29. The method of any one of claims 1-17, wherein said mammal is a human male weighing at least about 50 kg and has a GFR which is chronically less than about 40 ml/min.
30. The method of any one of claims 1-17, wherein said mammal is a human female weighing at least about 40 kg and has a GFR which is chronically less than about 30 ml/min.
31. The method of any one of claims 1-17, wherein said treatment or prevention reduces serum creatinine levels in said mammal by at least about 5% over 3 months.
32. The method of any one of claims 1-17, wherein prior to said treatment or prevention, said mammal presented a chronic decline in a clinical indicator of renal function, and after at least about 3 months of said treatment or prevention, said indicator stabilizes.
33. The method of any one of claims 1-6 and 12-17, wherein at least one of said ACEI, said AIIRA or said morphogen is administered orally, parenterally, intravenously, intraperitoneally, or into a renal capsule, or by an implanted device.

34. The method of claim 33, wherein a stent has been implanted into said mammal for said administration of at least one of said ACEI, said AIIRA or said morphogen.
35. The method of any one of claims 1-6 and 12-17, wherein at least one of said ACEI or said AIIRA, and at least one of said morphogen are conjointly administered at least once a week for a period of at least about one month.
36. The method of any one of claims 1-6 and 12-17, wherein at least one of said ACEI or AIIRA, and at least one of said morphogen are conjointly administered at least once a week for a period of at least about one year.
37. The method of any one of claims 1-6 and 12-17, wherein said ACEI or said AIIRA, and said morphogen are administered through different routes.
38. The method of any one of claims 1-6 and 12-17, wherein said ACEI or said AIIRA, and said morphogen are conjointly administered at different frequencies.
39. The method of any one of claims 1-6 and 12-17, wherein said morphogen is administered at a dosage of about 0.01-1000 µg/kg body weight of said mammal.
40. The method of claim 39, wherein said morphogen is administered at a dosage of about 10-300 µg/kg body weight of said mammal.
41. The method of any one of claims 1, 3, 5, 12, 14 and 16, wherein said ACEI is administered orally at a concentration of about 1-10,000 mg/L, preferably 10-1000 mg/L, 10-100 mg/L, 100-1000 mg/L, most preferably 100 mg/L.
42. The method of any one of claims 2, 4, 6, 13, 15 and 17, wherein said AIIRA is administered orally at a concentration of about 0.01-100 mg/kg body weight, preferably 0.1-10 mg/kg body weight, 0.2-5 mg/kg body weight, 0.5-2 mg/kg body weight, most preferably 1 mg/kg body weight.

43. The method of any one of claims 1-6 and 12-17, wherein said OP/BMP morphogen and, ACEI or AIIRA are administered in a single pharmaceutical composition.
44. The method of any one of claims 1-6 and 12-17, wherein said OP/BMP morphogen and, ACEI or AIIRA are administered in separate pharmaceutical compositions at or around the same time.
45. The method of any one of claims 1-6 and 12-17, wherein said OP/BMP morphogen and, ACEI or AIIRA are administered in separate pharmaceutical compositions at different times.
46. The method of any one of claims 1-17, wherein said morphogen (a) induces chondrogenesis in an ectopic bone assay; (b) prevents, inhibits, delays or alleviates loss of renal function in an animal model of chronic renal failure, or (c) causes a clinically significant improvement in a standard marker of renal function when administered to a mammal in, or at risk of, chronic renal failure.
47. The method of of any one of claims 1-17, wherein said morphogen comprises a polypeptide including at least a C-terminal cysteine domain of a protein selected from: a pro form, a mature form, or a soluble form of a polypeptide, wherein said polypeptide is: OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, or BMP9.
48. The method of claim 47, wherein said morphogen comprises a polypeptide including at least a C-terminal cysteine domain of a polypeptide selected from: a pro form, a mature form, or a soluble form of human OP-1.
49. The method of claim 1, wherein said morphogen comprises a polypeptide having at least 70% homology or 50% identity with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-1 (SEQ ID NO: 2).

50. The method of claim 49, wherein said polypeptide has at least 75% homology or 60% identity with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-1 (SEQ ID NO: 2).
51. The method of claim 49, wherein said polypeptide has at least 80% homology or 70% identity with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-1 (SEQ ID NO: 2).
52. The method of claim 53, wherein said polypeptide has at least 90% identity with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-1 (SEQ ID NO: 2).
53. The method of any one of claims 1, 3, 5, 7, 9-12, 14, and 16, wherein said ACEI is: any one compound of the formulas I-XXVIII or their salts thereof; acylmercapto and mercaptoalkanoyl prolines; captopril (1-[(2S)-3-mercaptop-2-methylpropionyl]-L-proline); ether or thioether mercaptoacyl prolines; zofenopril; carboxyalkyl dipeptides; enalapril (N-(1-ethoxycarbonyl-3-phenylpropyl)-L-ananyl-L-proline); lisinopril; quinapril; ramipril; carboxyalkyl dipeptide mimics; cilazapril; benazapril; phosphinylalkanoyl prolines; fosinopril; trandolopril; phosphonamide substituted amino or imino acids; phosphonate substituted amino or imino acids and salts thereof; ceronapril ((S)-1-[6-amino-2-[[hydroxyl(4-phenylbutyl)phosphinyl]oxy]-1-oxohexyl]-L-proline); BRL 36,378; MC-838; CGS 14824 (3-([1-ethoxycarbonyl-3-phenyl-(1S)-propyl]-amino)-2,3,4,5-tetrahydro-2-oxo-1-(3S)-benzazepine-1 acetic acid HCL); CGS 16,617 (3(S)-[[1S)-5-amino-1-carboxypentyl]amino]2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-1-ethanoic acid); Cetapril (alacepril, Dainippon); Ru 44570; Cilazapril; Ro 31-2201; Lisinopril; Indalapril (delapril); Rentiapril (fentiapril, Santen); Indolapril; Spirapril; Perindopril; Quinapril; CI 925 ([3S-[2[R(\*)]R(\*)]]3R(\*)]-2-[2-[[1-(ethoxy-carbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid HCL); WY-44221; mercapto-containing compounds; pivopril; YS980; Omapatrilat; Alacepril;

moveltopril; quinaprilat; moexipril; perinodpril (S-9490); pentopril; ancovenin; phenacein; or nicotianamin.

54. The method of any one of claims 2, 4, 6, 8-11, 13, 15, and 17, wherein said AIIRA is: Losartan (Cozaar®), Valsartan (Diovan®), Irbesartan (Avapro®), Candesartan (Atacand®), Telmisartan (Micardis®), tasosartan, zolarsartan, Teveten (eprosartan mesylate) or olmesartan medoxomil (Benicar).
55. The method of any one of claims 1, 3, 5, 7, 9-12, 14, and 16, wherein said ACEI is Enalapril.
56. A pharmaceutical composition comprising a therapeutically effective amount an ACE inhibitor and an OP/BMP morphogen formulated with pharmaceutically acceptable salt, carrier, excipient or diluent.
57. A pharmaceutical composition comprising a therapeutically effective amount an AIIRA and an OP/BMP morphogen formulated with pharmaceutically acceptable salt, carrier, excipient or diluent.
58. The pharmaceutical composition of claim 56, wherein the ACE inhibitor is Enalapril.
59. The pharmaceutical composition of claim 57, wherein the AIIRA is: Losartan (Cozaar®), Valsartan (Diovan®), Irbesartan (Avapro®), Candesartan (Atacand®), Telmisartan (Micardis®), tasosartan, zolarsartan, Teveten (eprosartan mesylate) or olmesartan medoxomil (Benicar).
60. The pharmaceutical composition of claim 56 or 57, wherein the morphogen is the polypeptide of SEQ ID NO: 3.
61. The pharmaceutical composition of claim 56 or 57, wherein the morphogen is a first polypeptide including at least a C-terminal cysteine domain of a protein selected from: a pro form, a mature form, or a soluble form of a second polypeptide, wherein said second polypeptide is: OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, or BMP9.

62. The pharmaceutical composition of claim 56 or 57, wherein said morphogen comprises a polypeptide having at least 70% homology or 50% identity with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-1 (SEQ ID NO: 2).
63. The pharmaceutical composition of claim 62, wherein said polypeptide has at least 75% homology or 60% identity with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-1 (SEQ ID NO: 2).
64. The pharmaceutical composition of claim 62, wherein said polypeptide has at least 80% homology or 70% identity with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-1 (SEQ ID NO: 2).
65. The pharmaceutical composition of claim 62, wherein said polypeptide has at least 90% identity with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-1 (SEQ ID NO: 2).
66. The pharmaceutical composition of claim 56, wherein said ACEI is: any one compound of the formulas I-XXVIII or their salts thereof; acylmercapto and mercaptoalkanoyl prolines; captopril (1-[(2S)-3-mercaptop-2-methylpropionyl]-L-proline); ether or thioether mercaptoacyl prolines; zofenopril; carboxyalkyl dipeptides; enalapril (N-(1-ethoxycarbonyl-3-phenylpropyl)-L-ananyl-L-proline); lisinopril; quinapril; ramipril; carboxyalkyl dipeptide mimics; cilazapril; benazapril; phosphinylalkanoyl prolines; fosinopril; trandolopril; phosphonamide substituted amino or imino acids; phosphonate substituted amino or imino acids and salts thereof; ceronapril ((S)-1-[6-amino-2-[[hydroxyl(4-phenylbutyl)phosphinyl]oxy]-1-oxohexyl]-L-proline); BRL 36,378; MC-838; CGS 14824 (3-([1-ethoxycarbonyl-3-phenyl-(1S)-propyl]-amino)-2,3,4,5-tetrahydro-2-oxo-1-(3S)-benzazepine-1 acetic acid HCL); CGS 16,617 (3(S)-[[1S)-5-amino-1-carboxypentyl]amino]2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-1-ethanoic acid); Cetapril (alacepril, Dainippon); Ru 44570; Cilazapril; Ro 31-2201; Lisinopril; Indalapril (delapril); Rentiapril (fentiapril, Santen); Indolapril; Spirapril; Perindopril; Quinapril; CI 925 ([3S-[2[R(\*)]R(\*)]]3R(\*)]-2-[2-[[1-

(ethoxy-carbonyl)-3-phenylpropyl]amino[-1-oxopropyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid HCL); WY-44221; mercapto-containing compounds; pivopril; YS980; Omapatrilat; Alacepril; moveltopril; quinaprilat; moexipril; perinodpril (S-9490); pentopril; ancovenin; phenacein; or nicotianamin.

67. The pharmaceutical composition of claim 57, wherein said AIIRA is: Losartan (Cozaar<sup>®</sup>), Valsartan (Diovan<sup>®</sup>), Irbesartan (Avapro<sup>®</sup>), Candesartan (Atacand<sup>®</sup>), Telmisartan (Micardis<sup>®</sup>), tasosartan, zolarsartan, Teveten (eprosartan mesylate) or olmesartan medoxomil (Benicar).
68. A package pharmaceutical comprising the pharmaceutical composition of any one of claims 56-67, in association with instructions for administering the composition to a mammal for treatment or prevention of chronic renal failure.

**Abstract**

The present invention provides reagents and methods for the treatment, and pharmaceuticals for use in the prevention and/or treatment, of chronic renal failure and other renal disorders in subjects (particularly mammalian subjects) renal replacement therapy. The methods involve the conjoint administration of ACE (Angiotensin-Converting Enzyme) inhibitors or Angiotensin II Receptor Antagonists (AIIRAs) with one or more OP/BMP family of proteins (morphogens, or inducers of morphogens, or agonists of the corresponding morphogen receptors, etc.). The invention also provides methods for implantation of renal cells induced with the conjoint administration of ACE inhibitors or AIIRAs with those morphogens.